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(19) **United States**(12) **Patent Application Publication****Yayon et al.**(10) **Pub. No.: US 2005/0147612 A1**(43) **Pub. Date: Jul. 7, 2005**(54) **ANTIBODIES THAT BLOCK RECEPTOR
PROTEIN TYROSINE KINASE ACTIVATION,
METHODS OF SCREENING FOR AND USES
THEREOF**(76) Inventors: **Avner Yayon**, Moshav Sitria (IL); **Eran
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WASHINGTON, DC 20006 (US)**(21) Appl. No.: **10/734,661**(22) Filed: **Dec. 15, 2003****Related U.S. Application Data**(63) Continuation of application No. PCT/IL02/00494,
filed on Jun. 20, 2002.(60) Provisional application No. 60/299,187, filed on Jun.
20, 2001.**Publication Classification**(51) **Int. Cl.⁷** **A61K 39/395**; C07H 21/04;
C07K 16/28; C07K 16/40;
C12N 5/06
(52) **U.S. Cl.** **424/146.1**; 530/388.22; 530/388.26;
435/69.1; 435/334; 435/320.1;
536/23.53(57) **ABSTRACT**

Molecules comprising the antigen-binding portion of antibodies that block constitutive and/or ligand-dependent activation of a receptor protein tyrosine kinase, such as fibroblast growth factor receptor **3** (FGFR3), are found through screening methods, where a soluble dimeric form of a receptor protein tyrosine kinase is used as target for screening a library of antibody fragments displayed on the surface of bacteriophage. The molecules of the present invention which block constitutive activation can be administered to treat or inhibit skeletal dysplasia, craniosynostosis disorders, cell proliferative diseases or disorders, or tumor progression associated with the constitutive activation of a receptor protein tyrosine kinase.

Figure 1

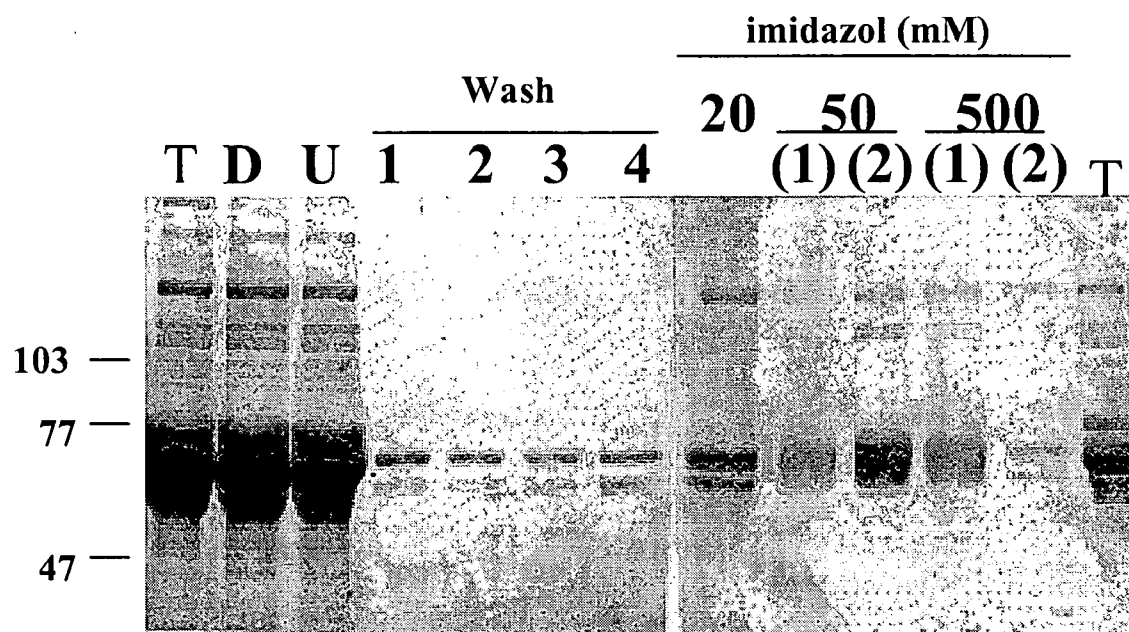


Figure 2

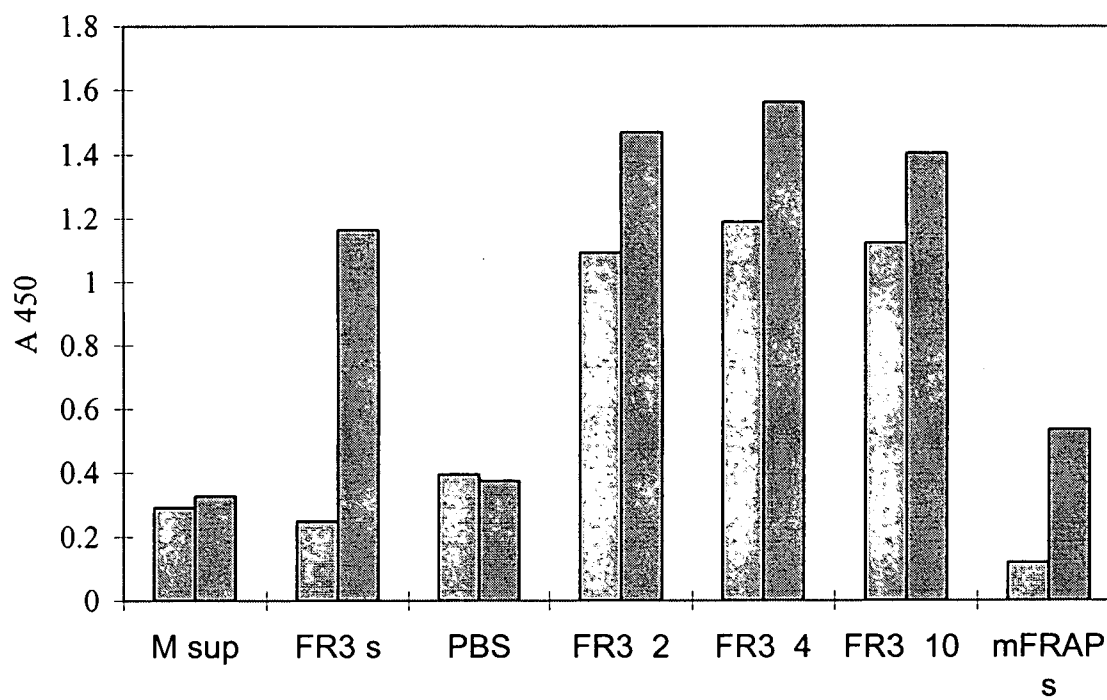


Figure 3

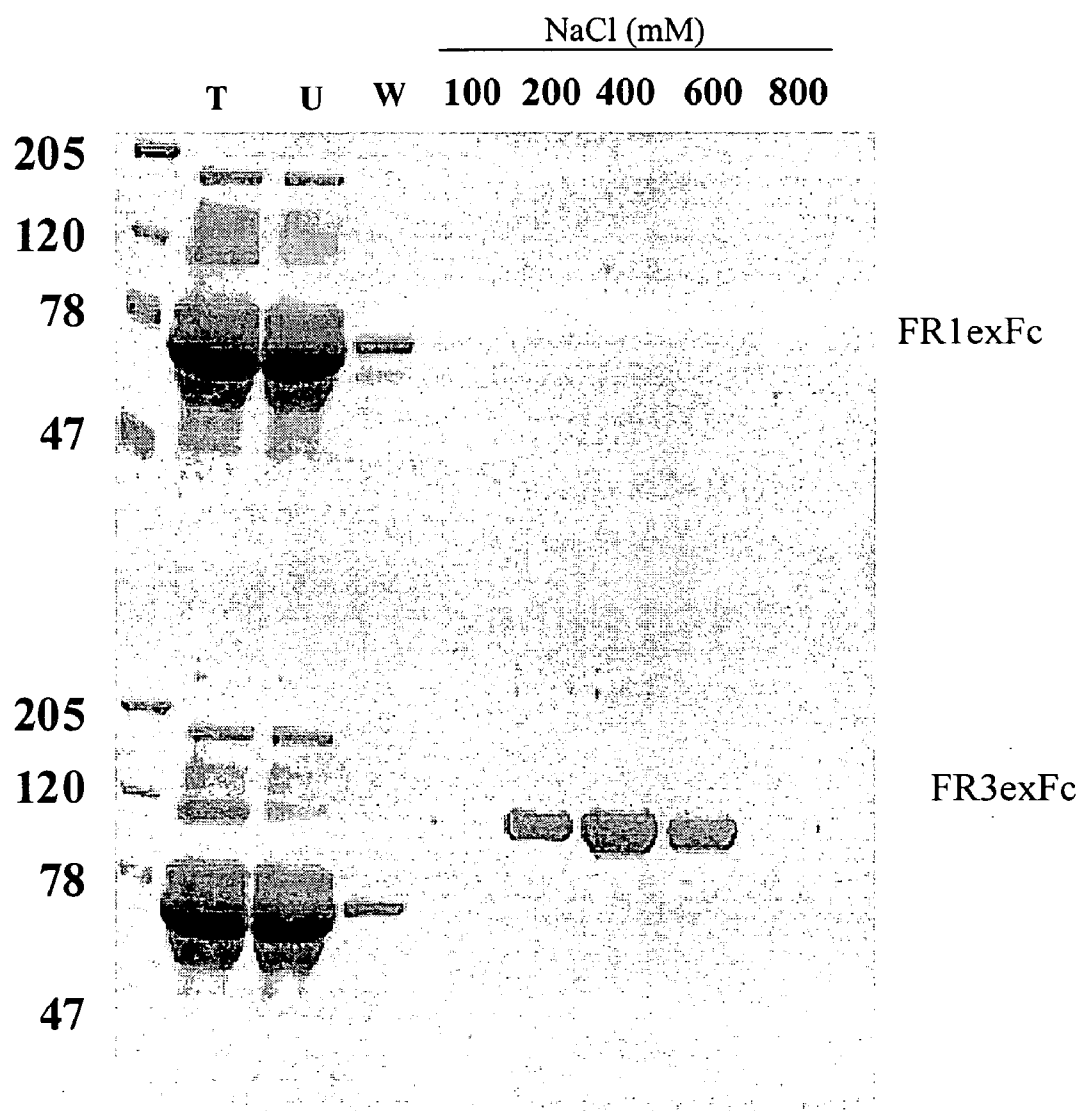


Figure 4

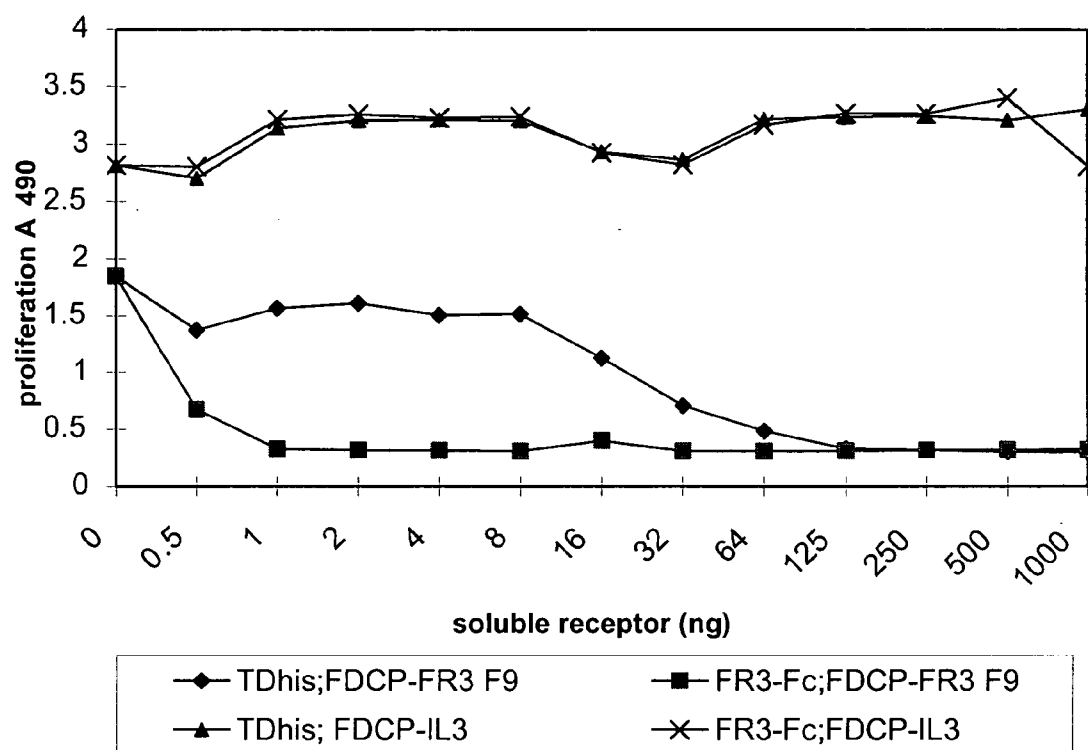


Figure 5

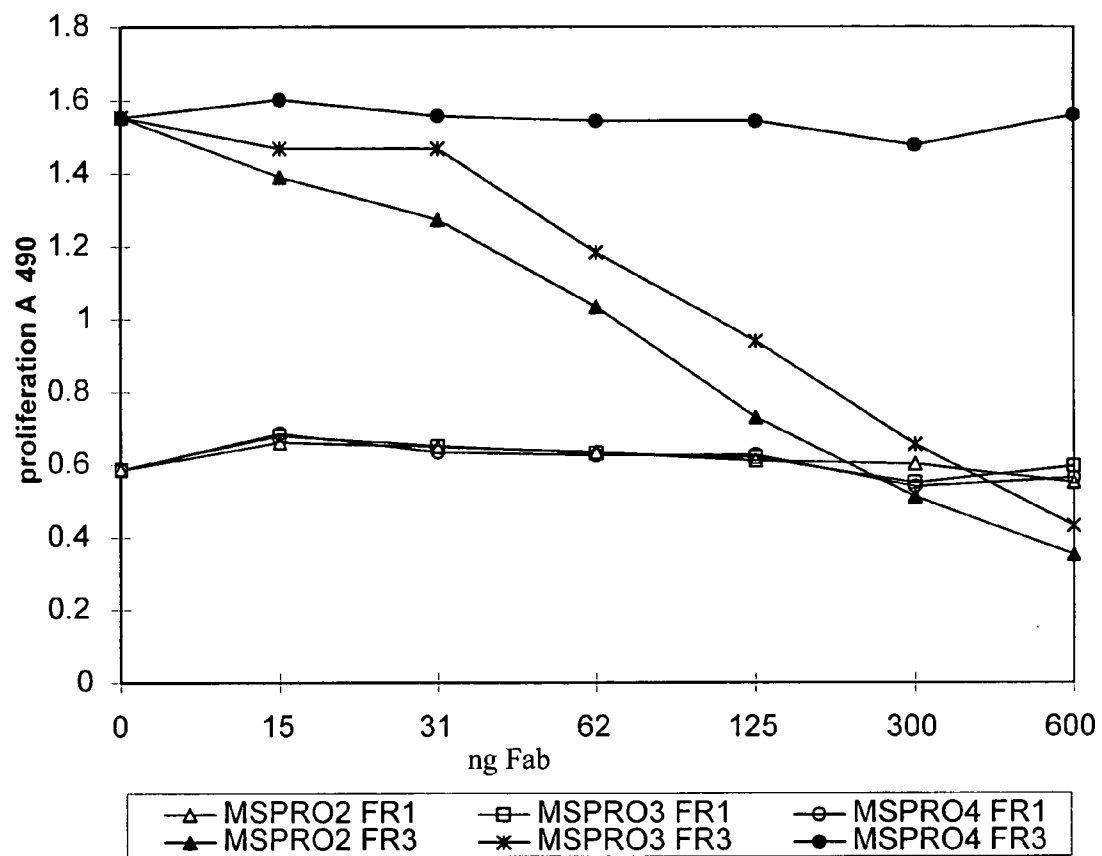


Figure 6

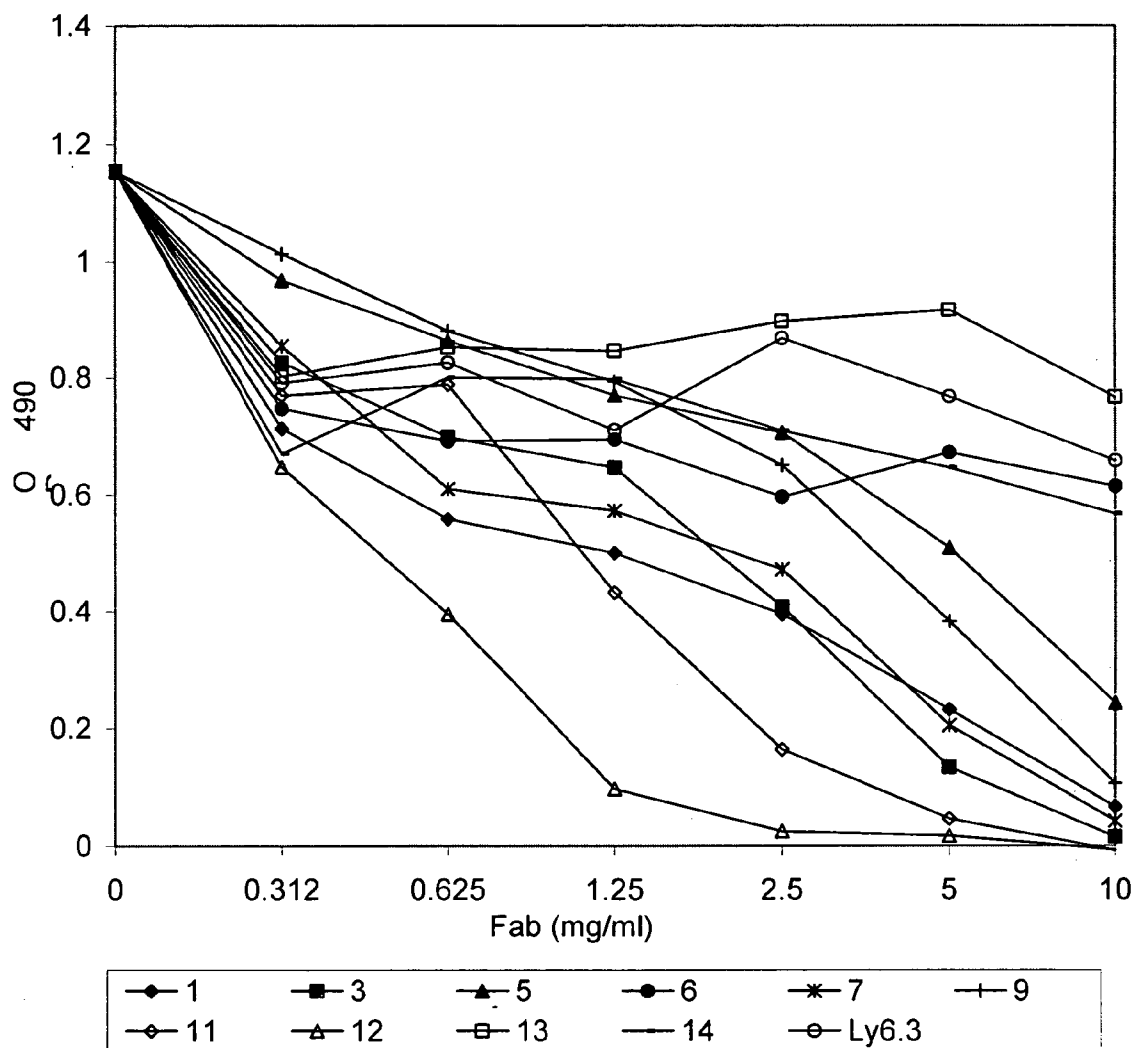


Figure 7A

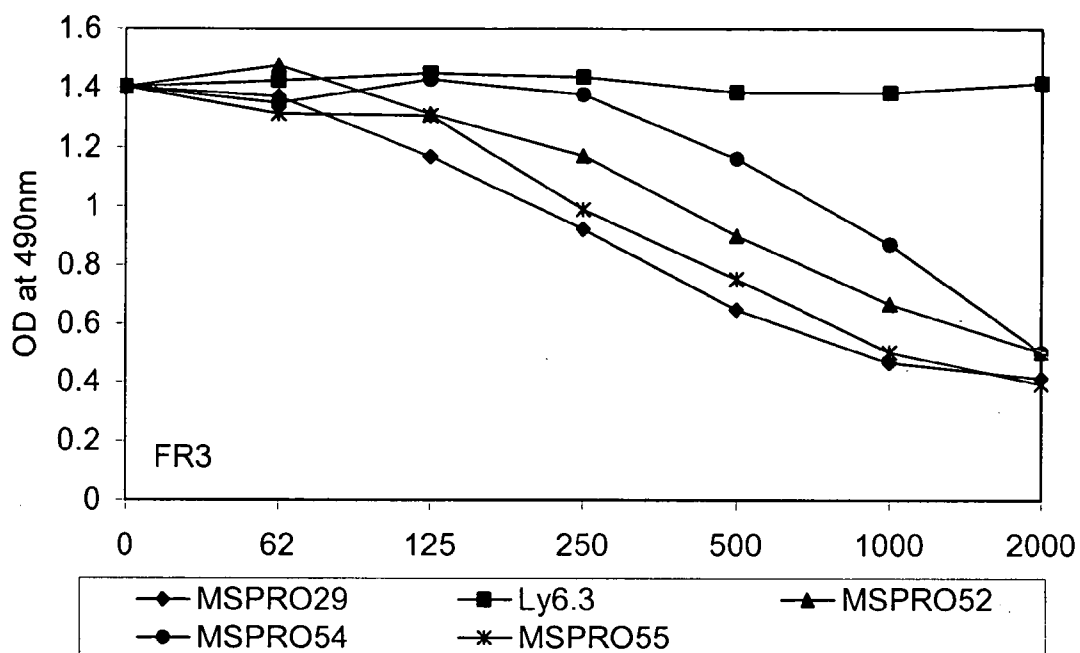


Figure 7B

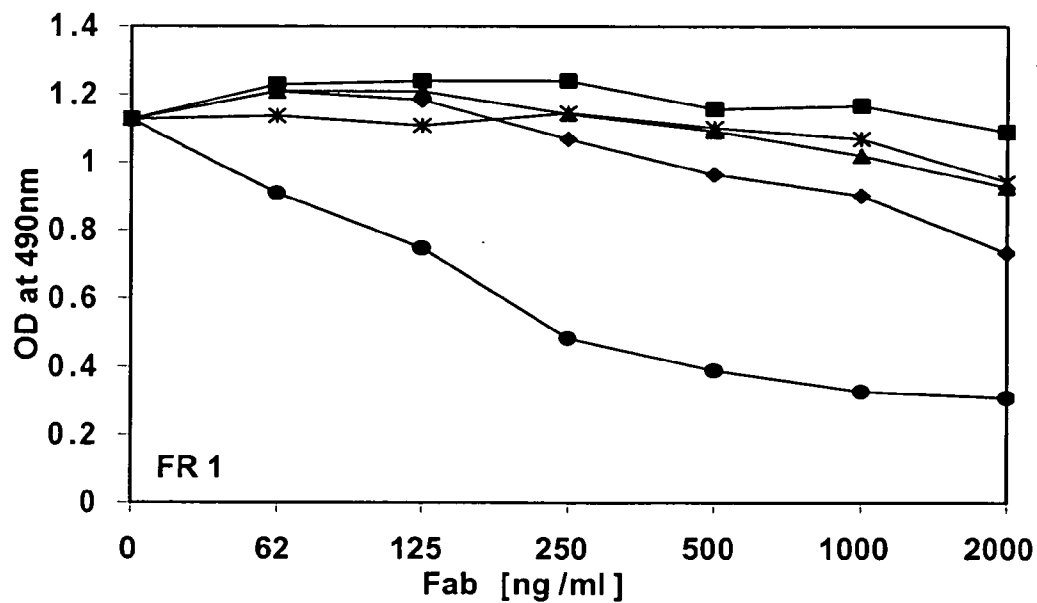


Figure 8A

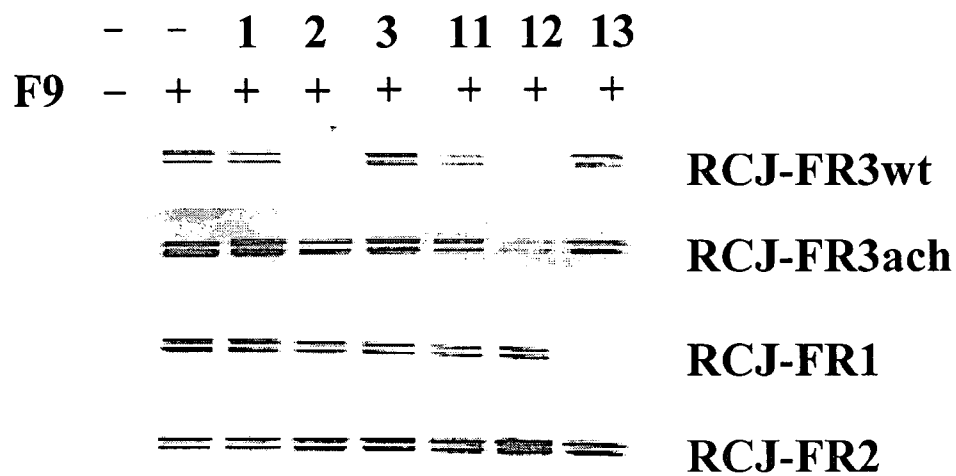


Figure 8B

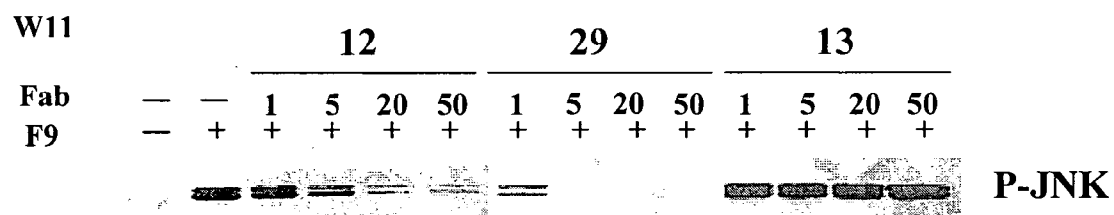


FIGURE 9A

M14

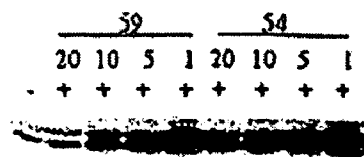
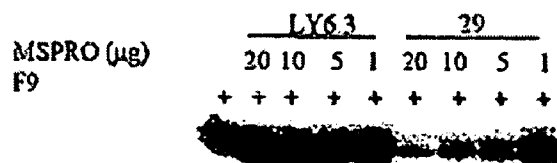


FIGURE 9B

W11

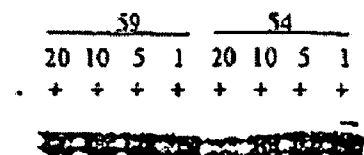
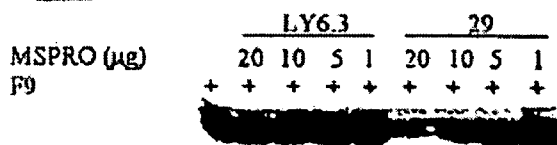


FIGURE 9C

R1-1

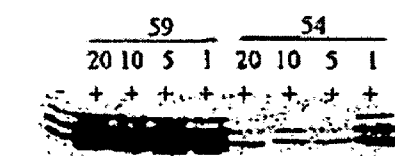
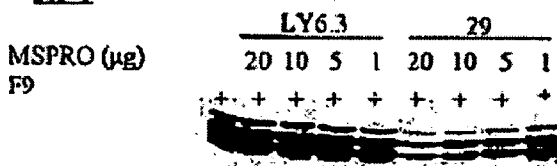


FIGURE 9D

R2-2

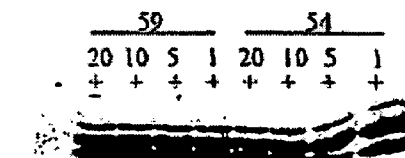
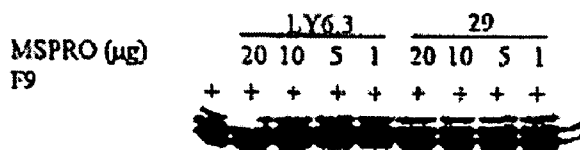


Figure 10

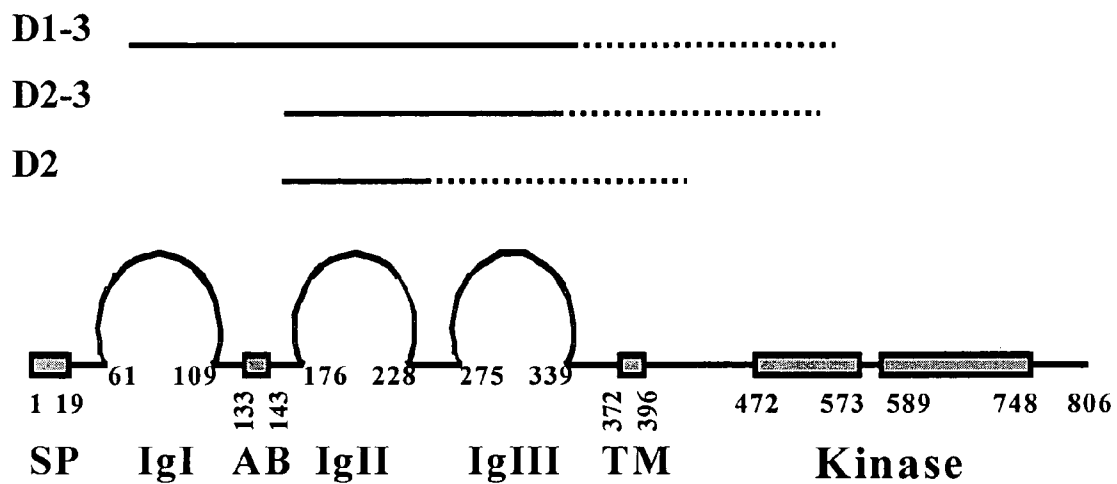


Figure 11

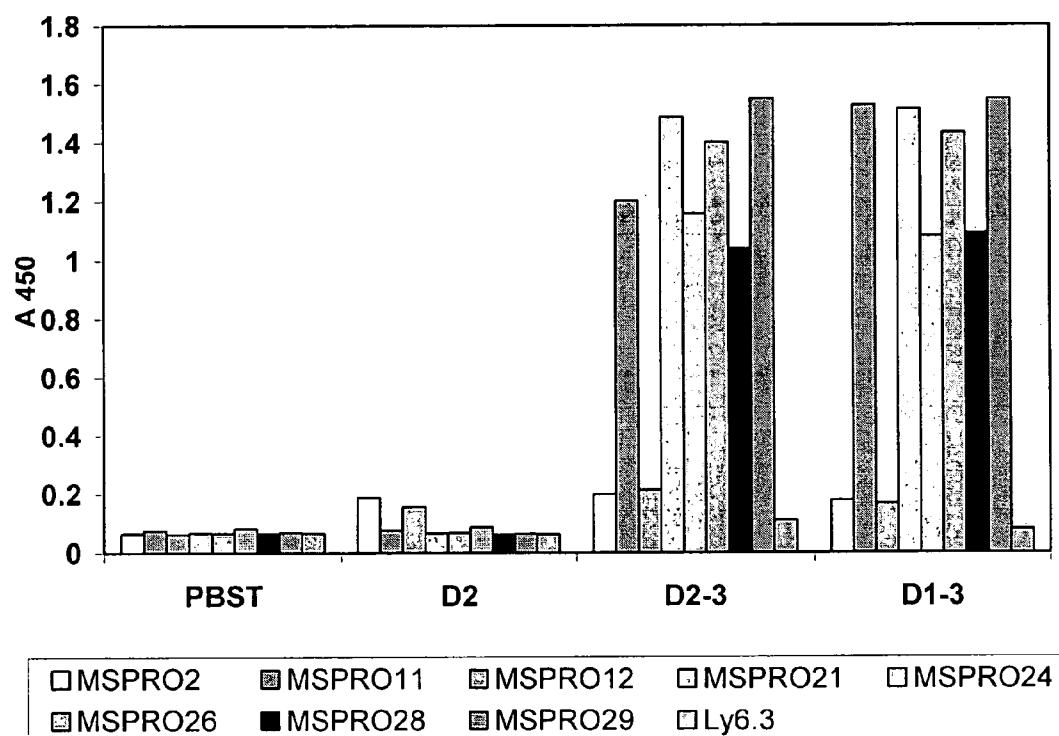


Figure 12

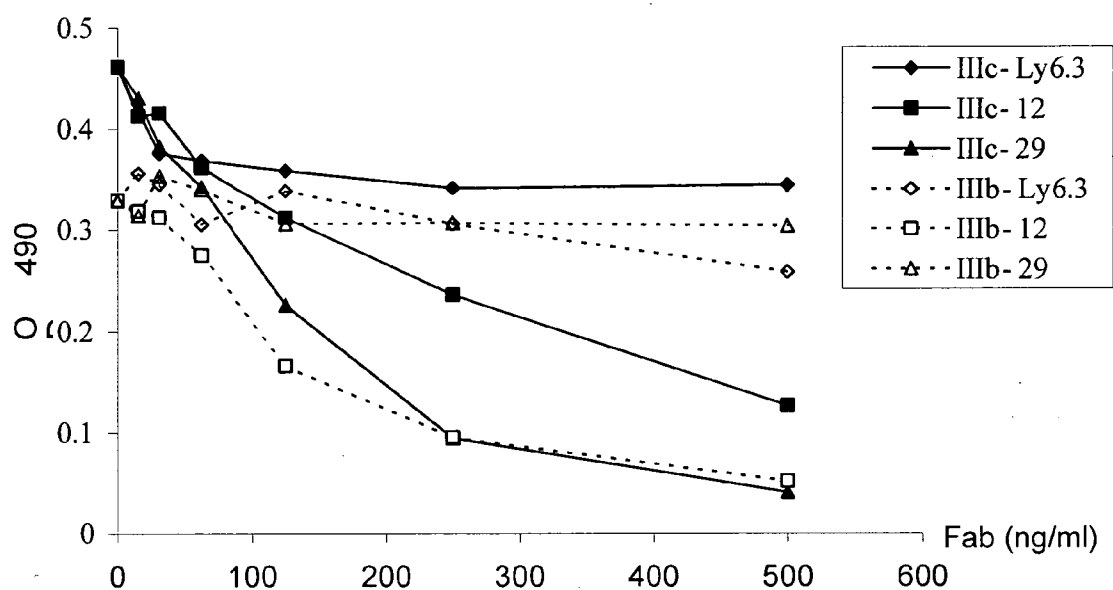
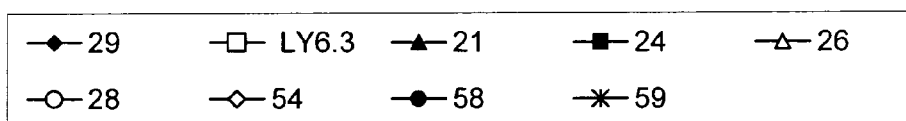
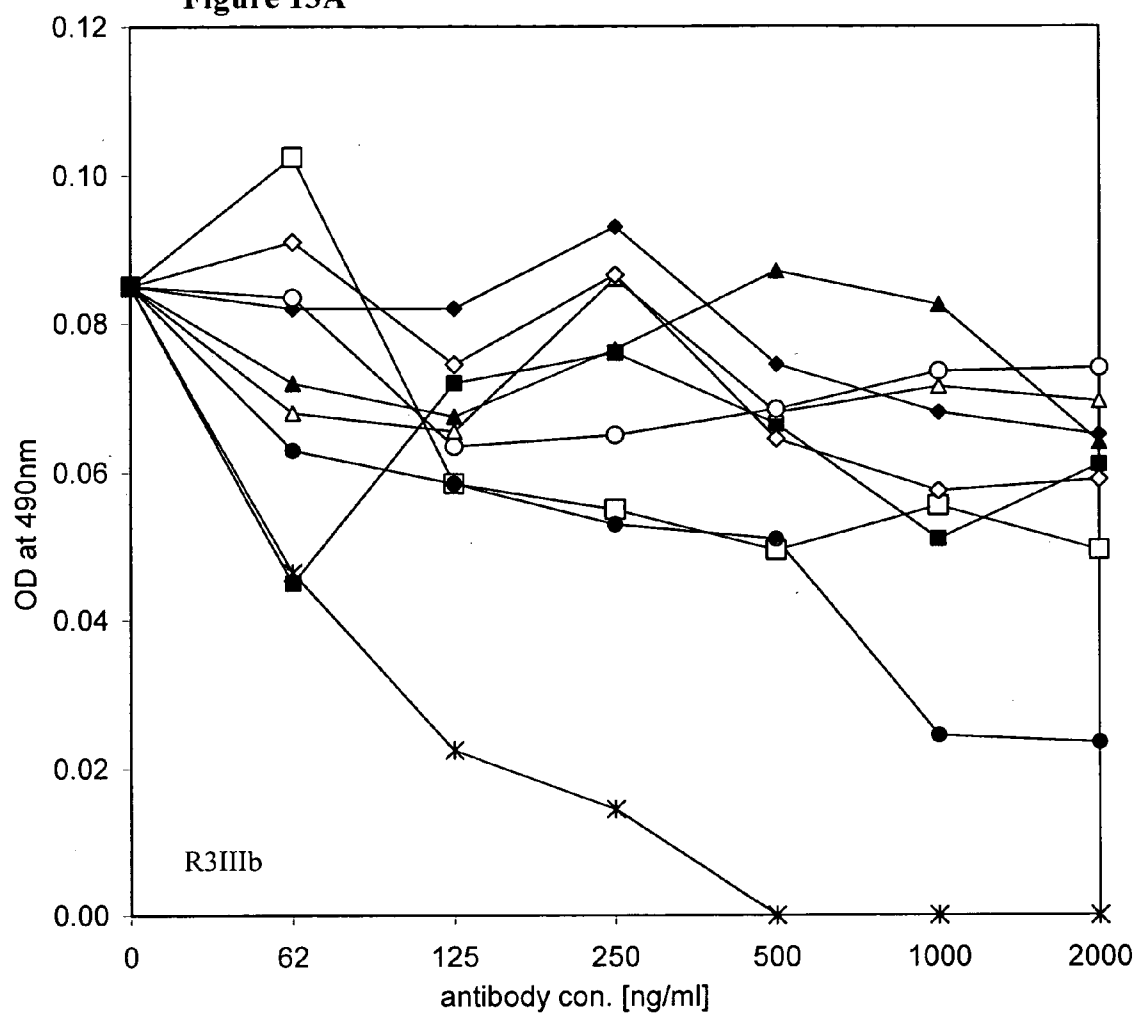


Figure 13A



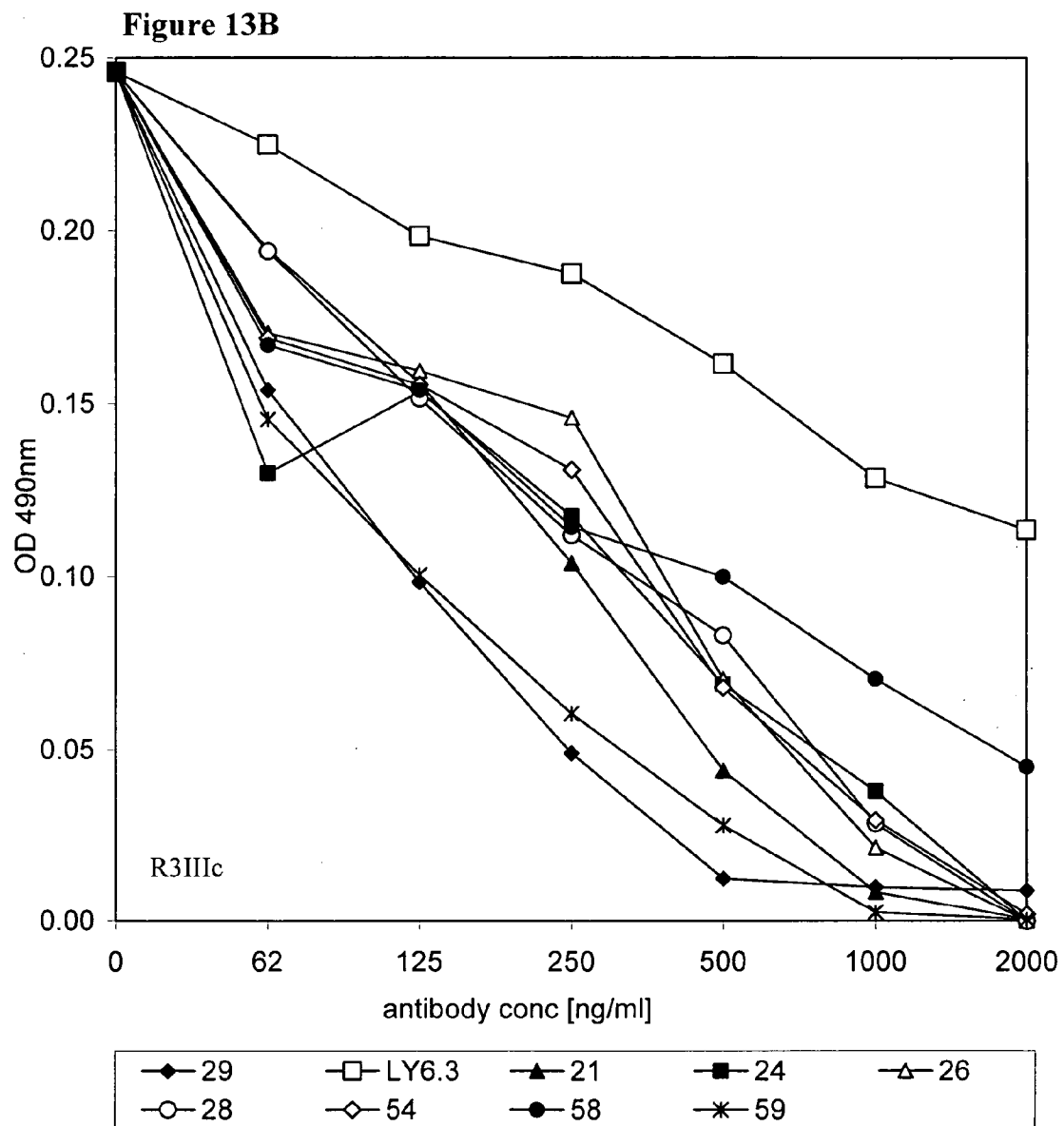


Figure 14

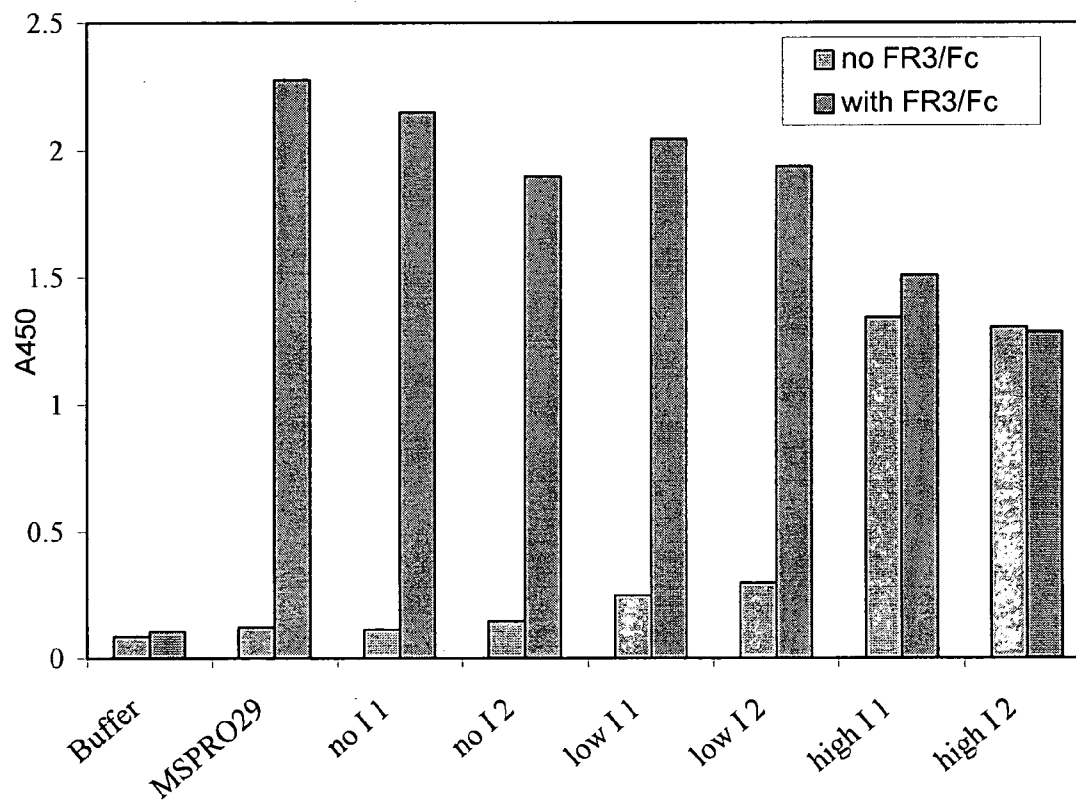
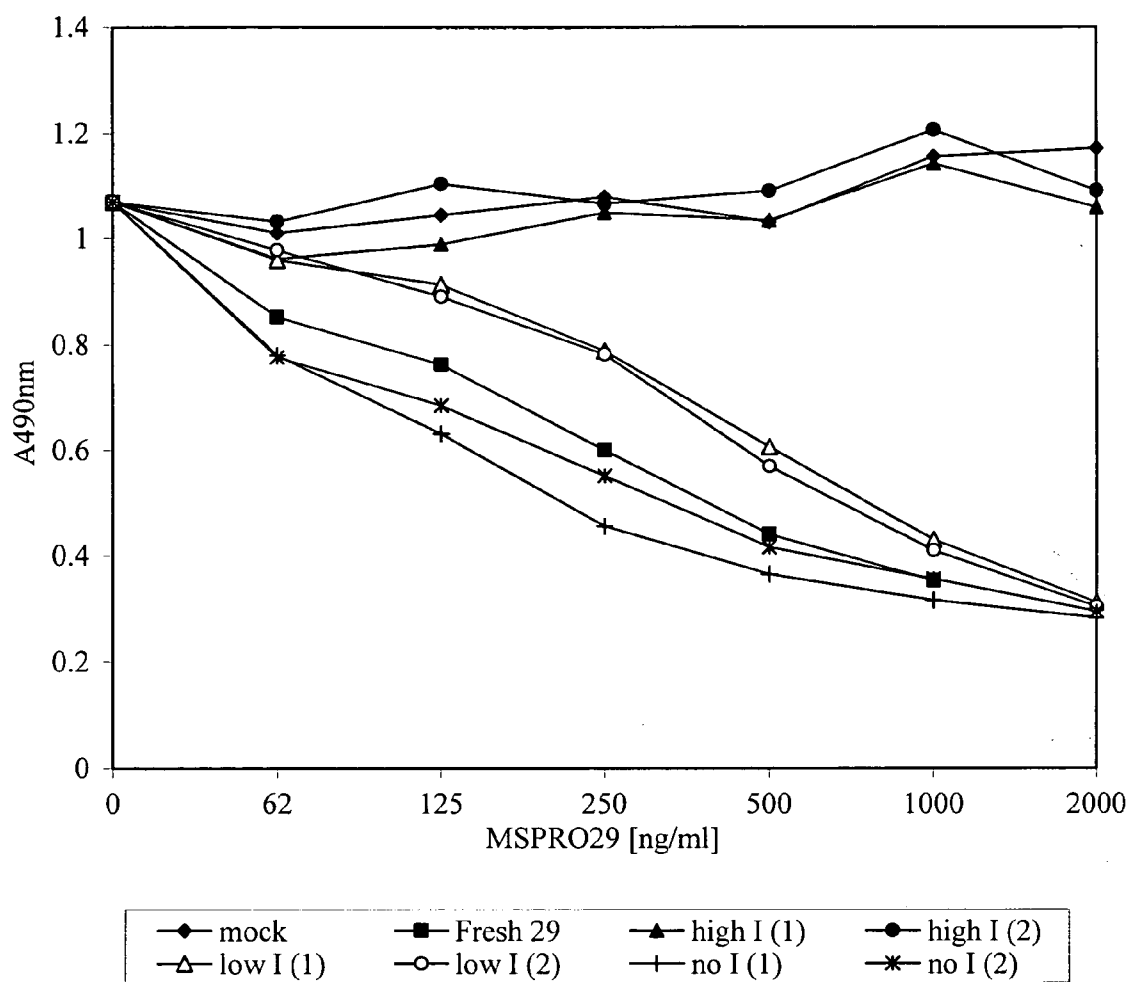


Figure 15



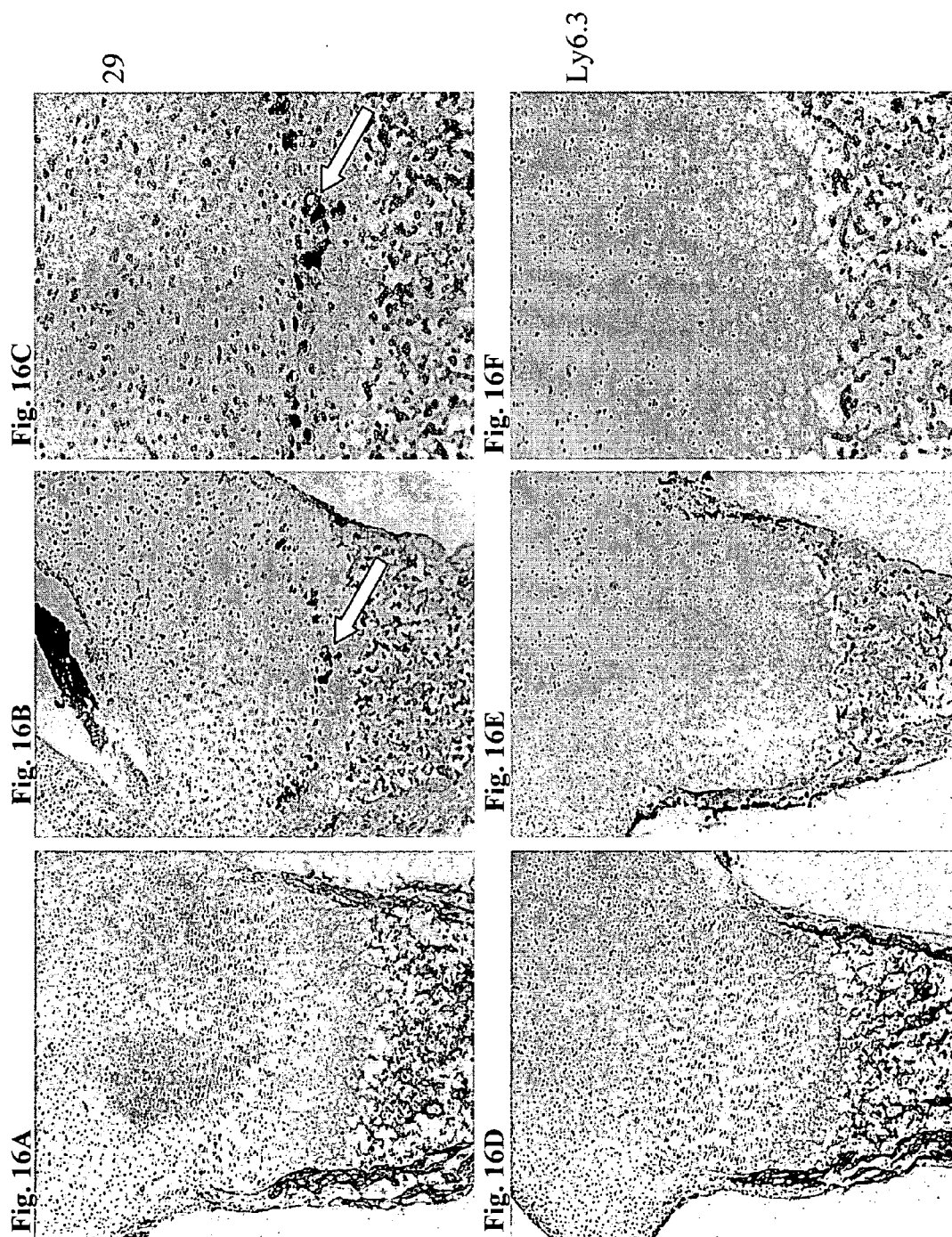


Figure 17A

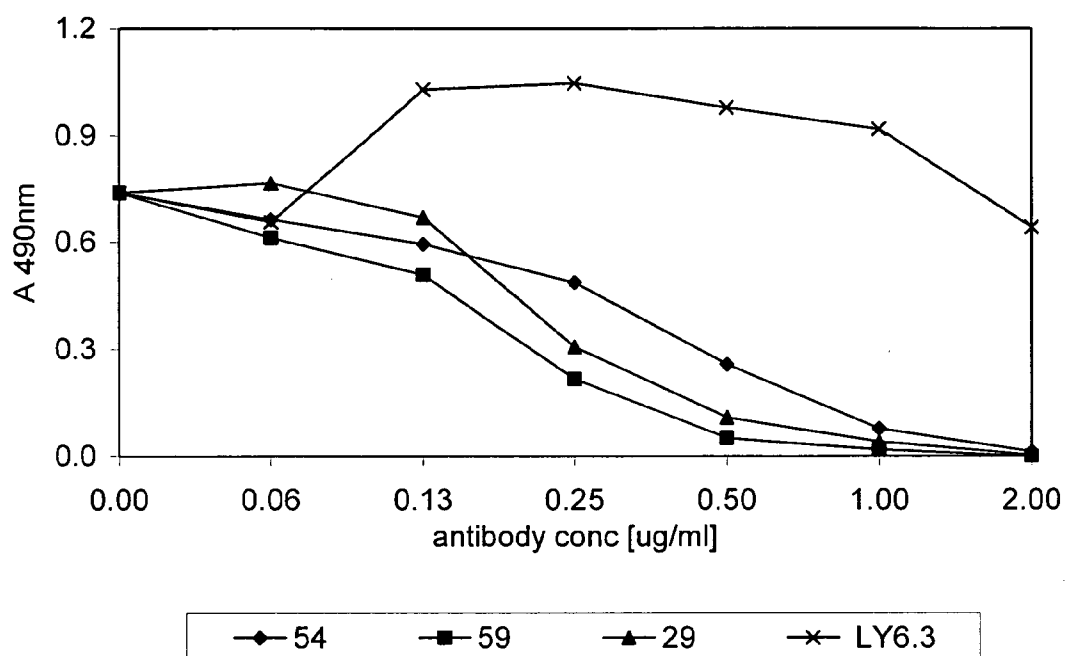


Figure 17B

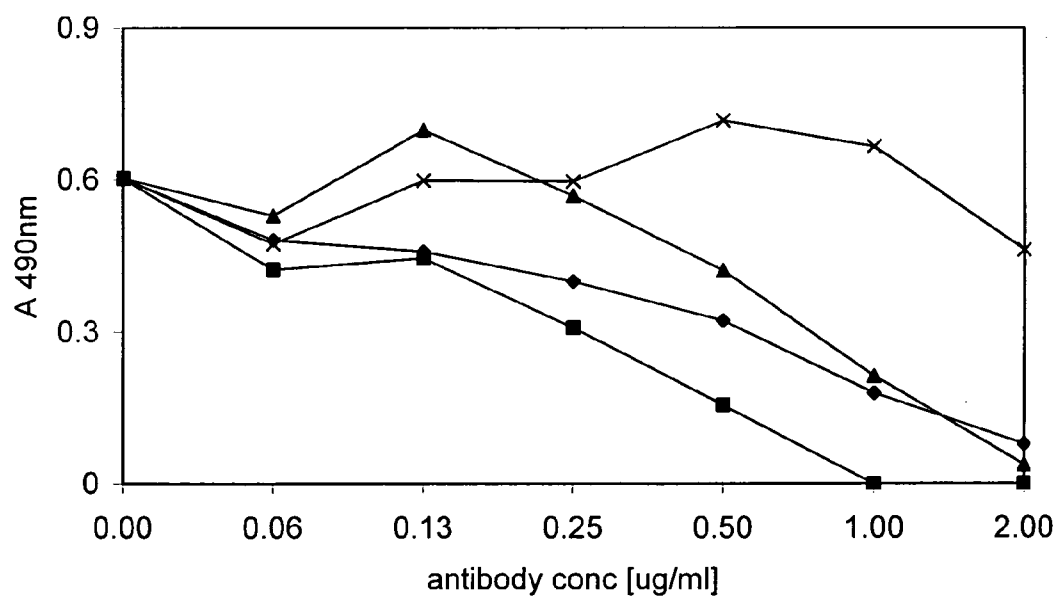


Figure 18A

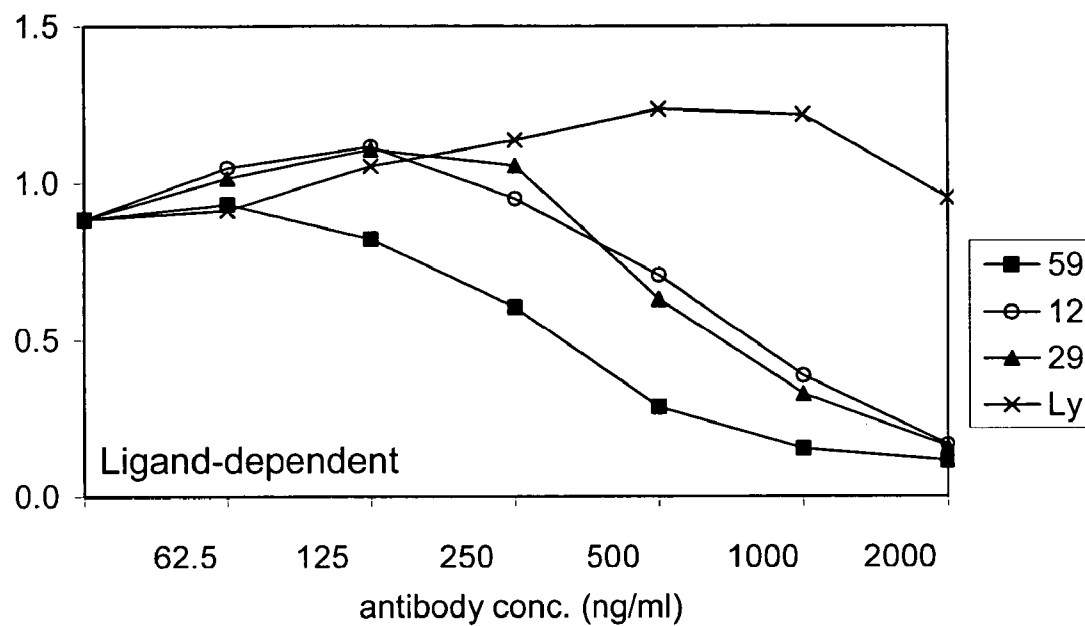


Figure 18B

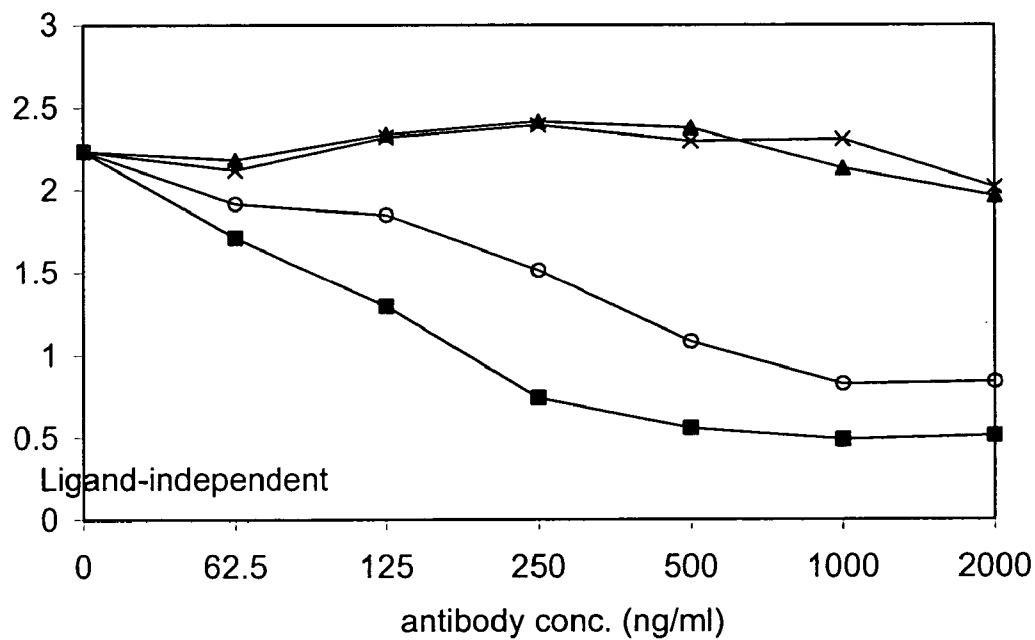


Figure 19

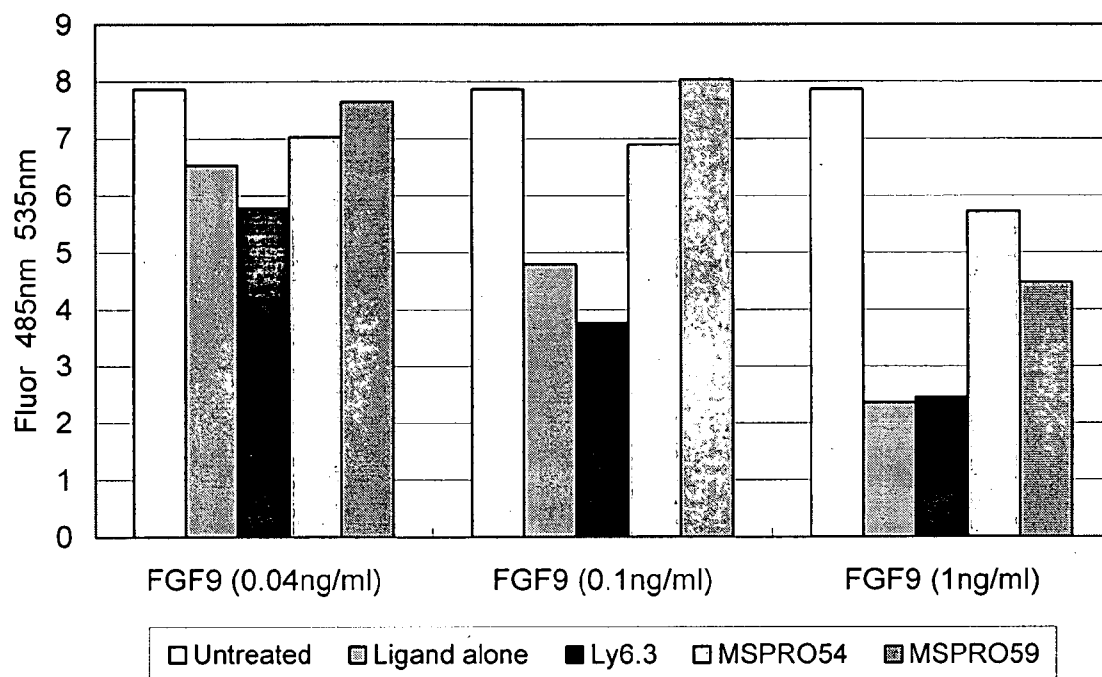


Figure 20

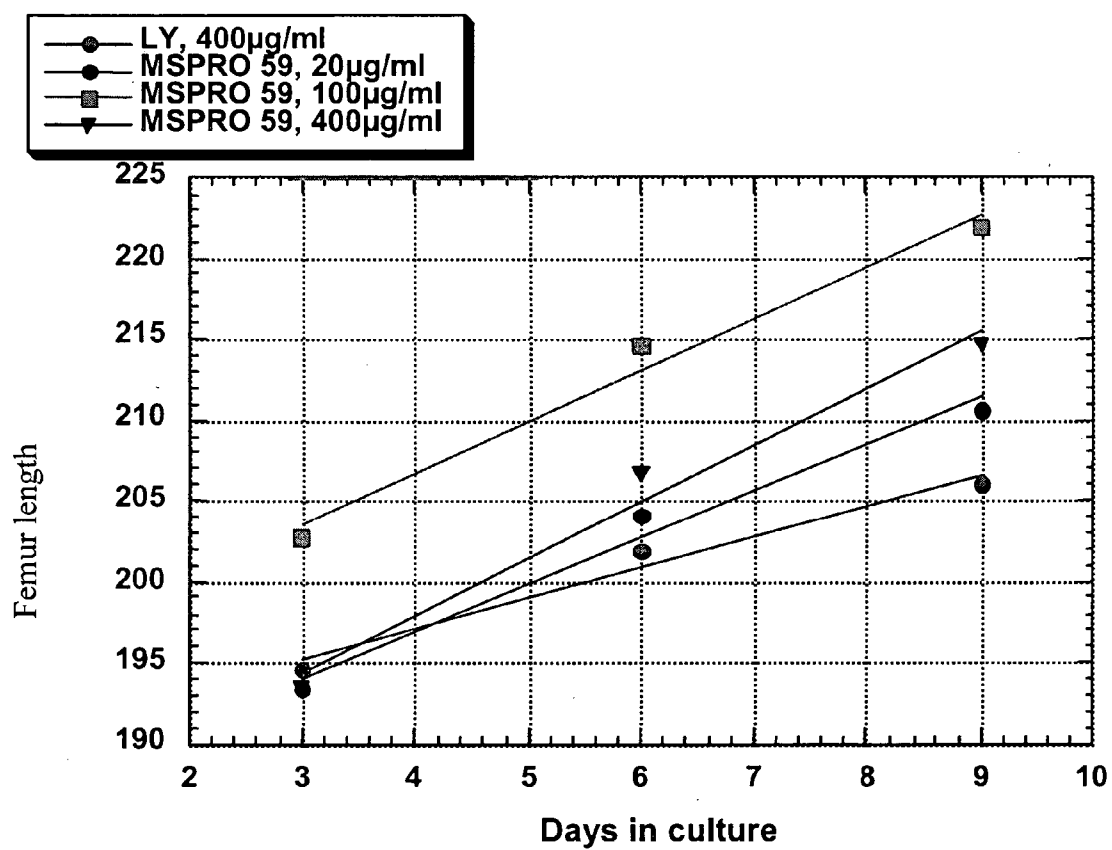


Figure 21

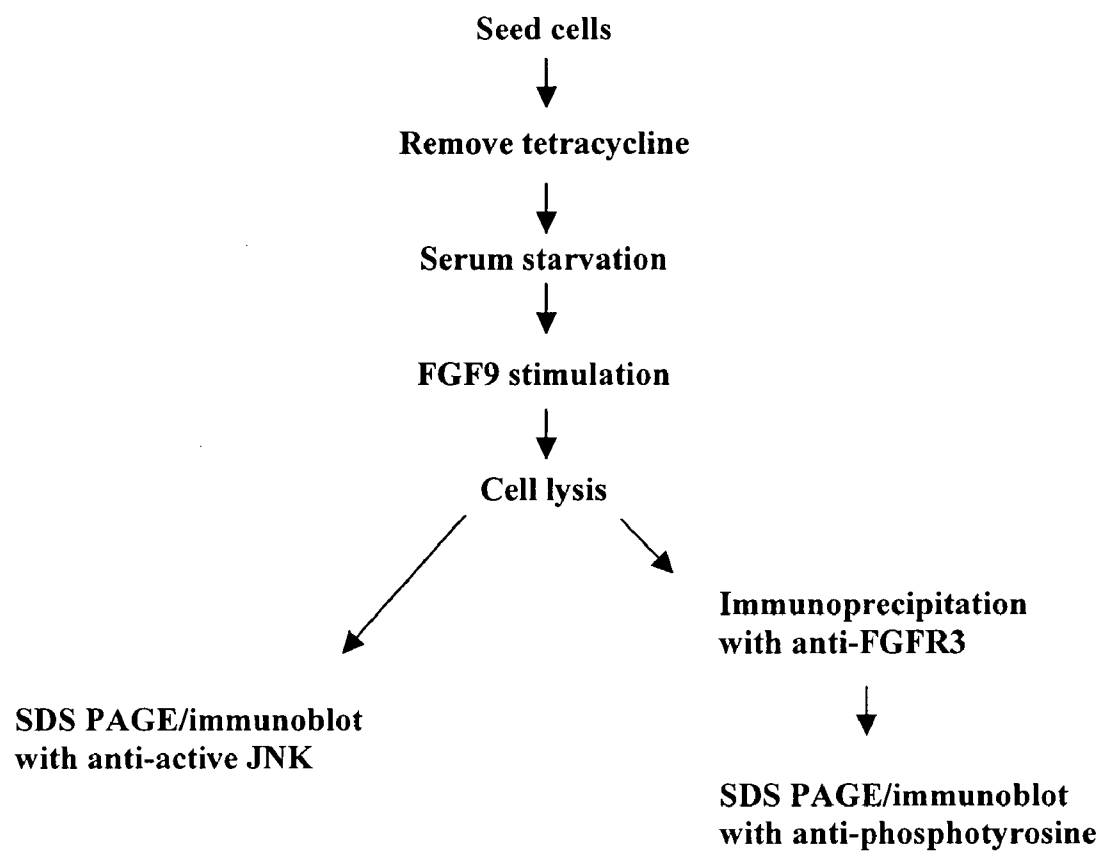


Figure 22

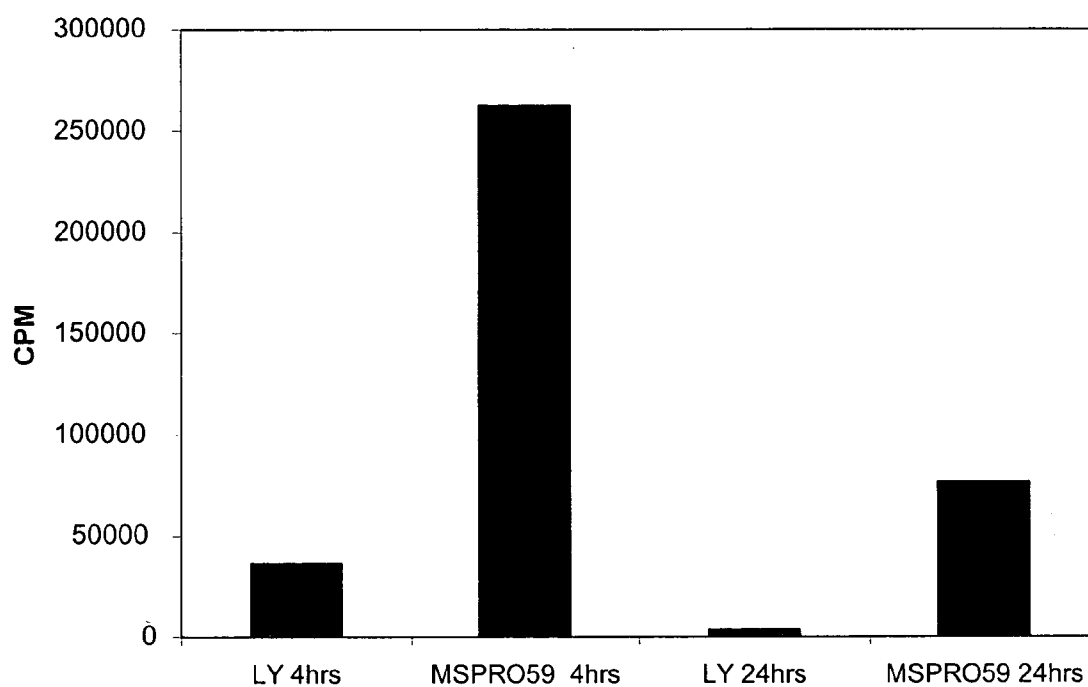


Figure 23

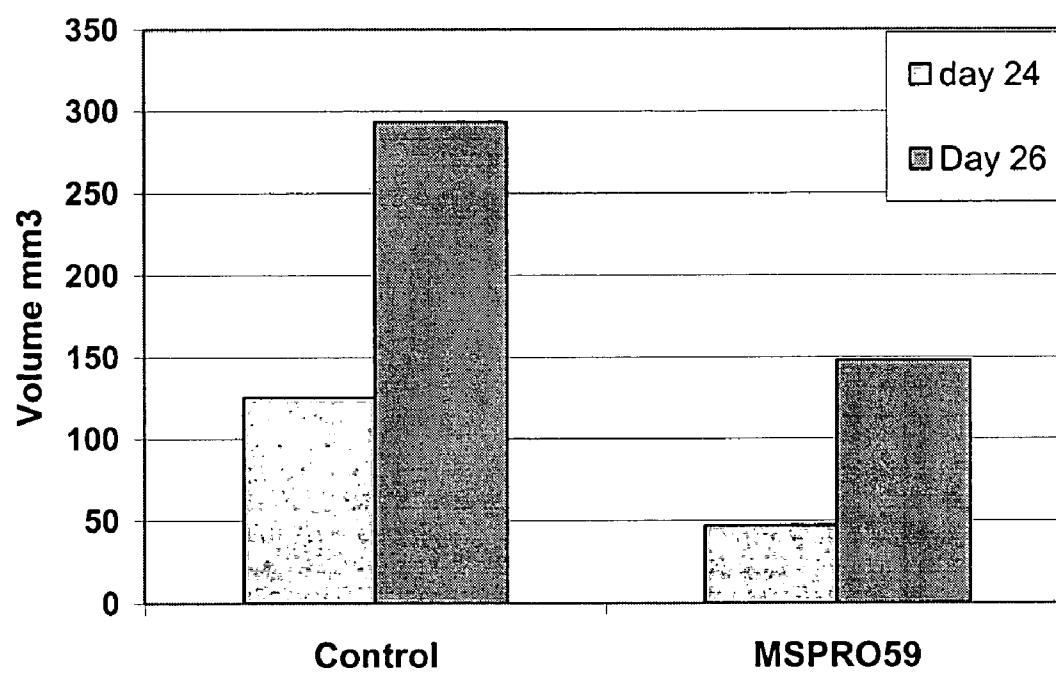


Figure 24

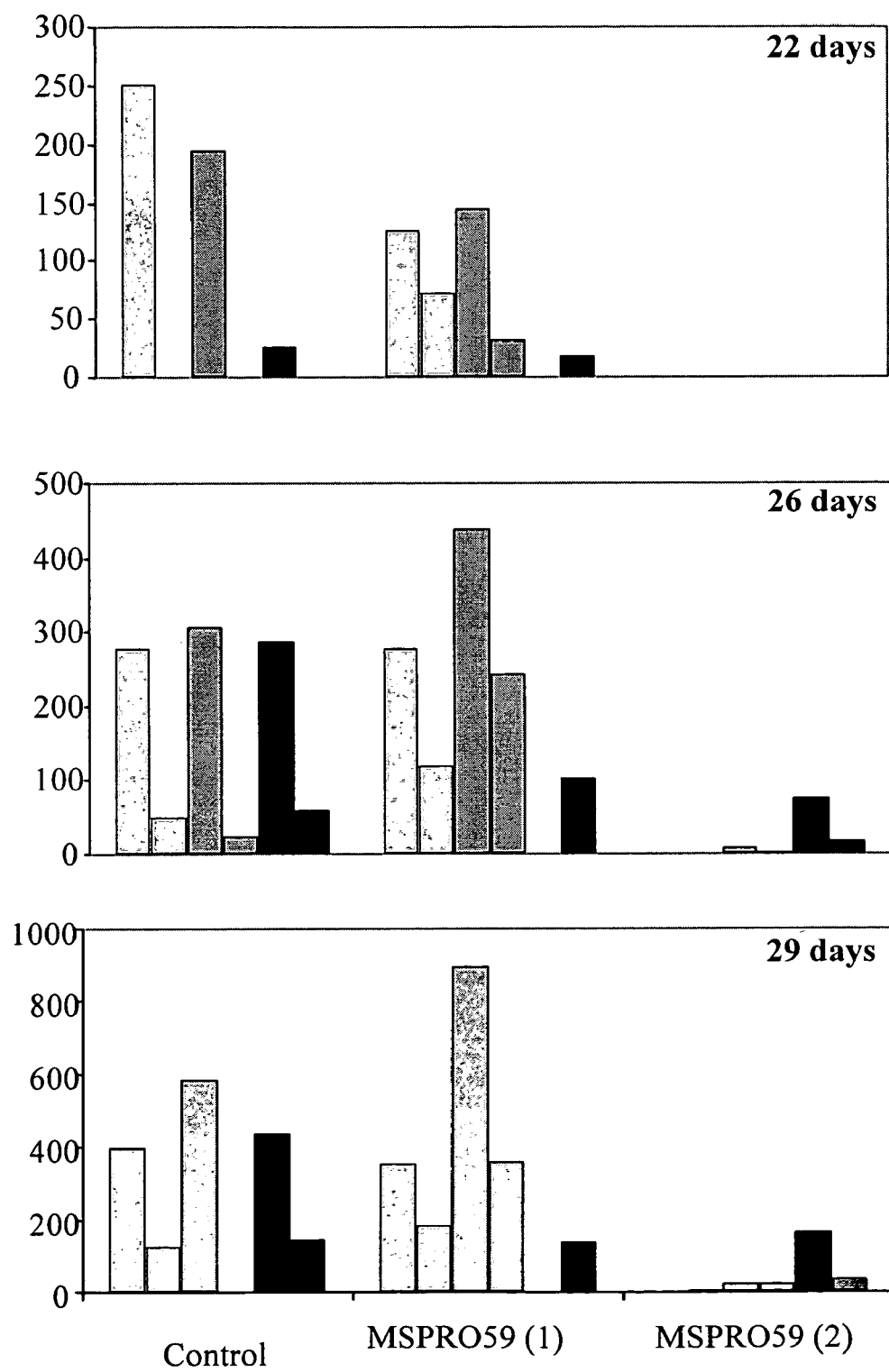


Figure 25A

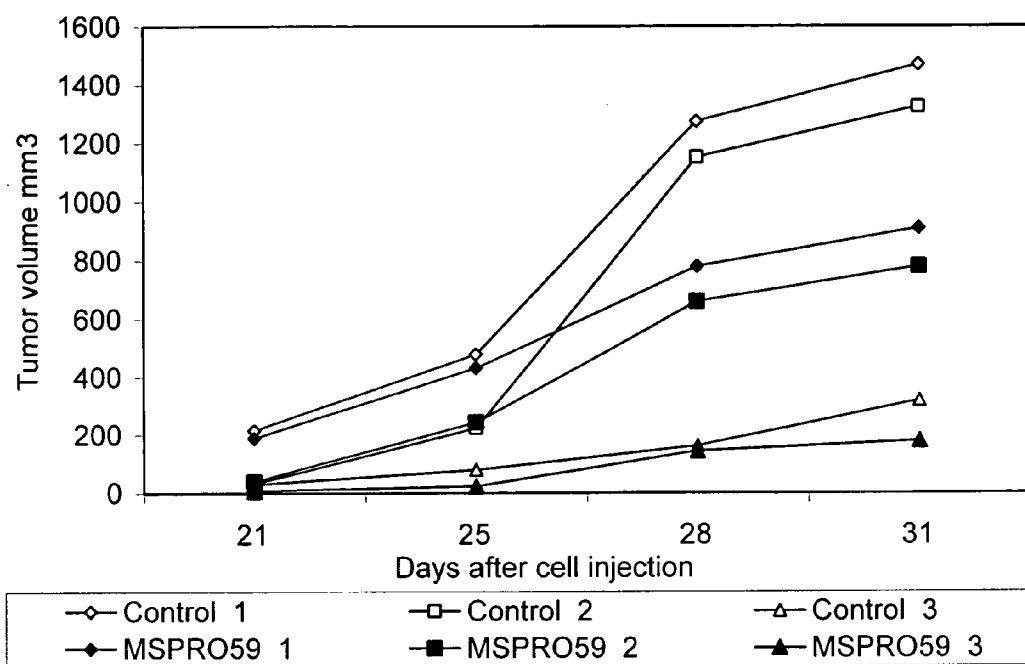


Figure 25B

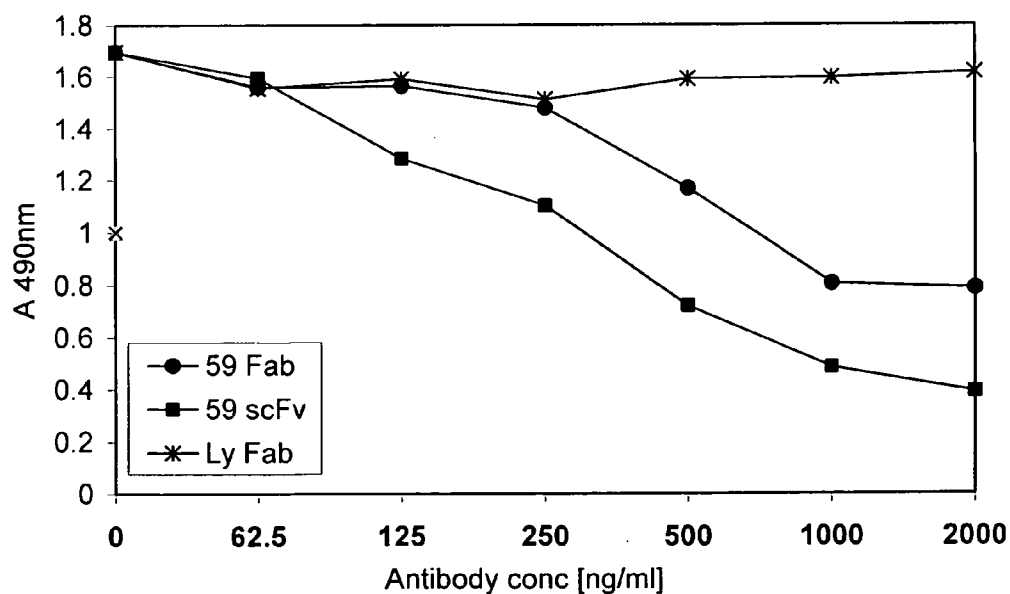


Figure 26

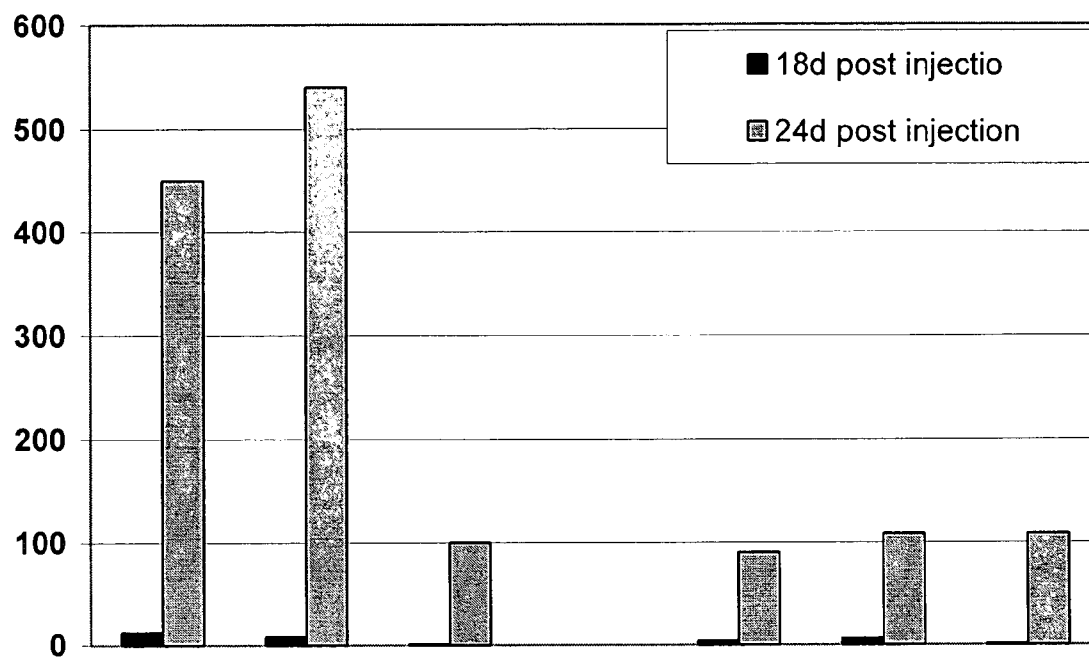


Figure 27

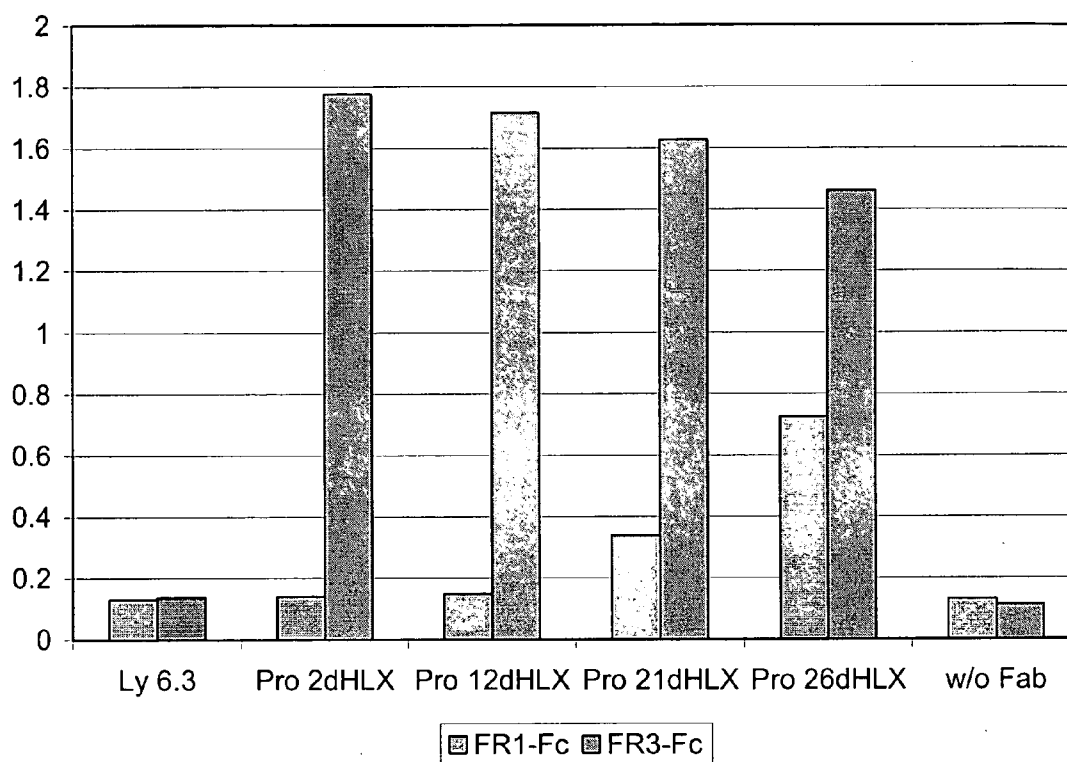


Figure 28

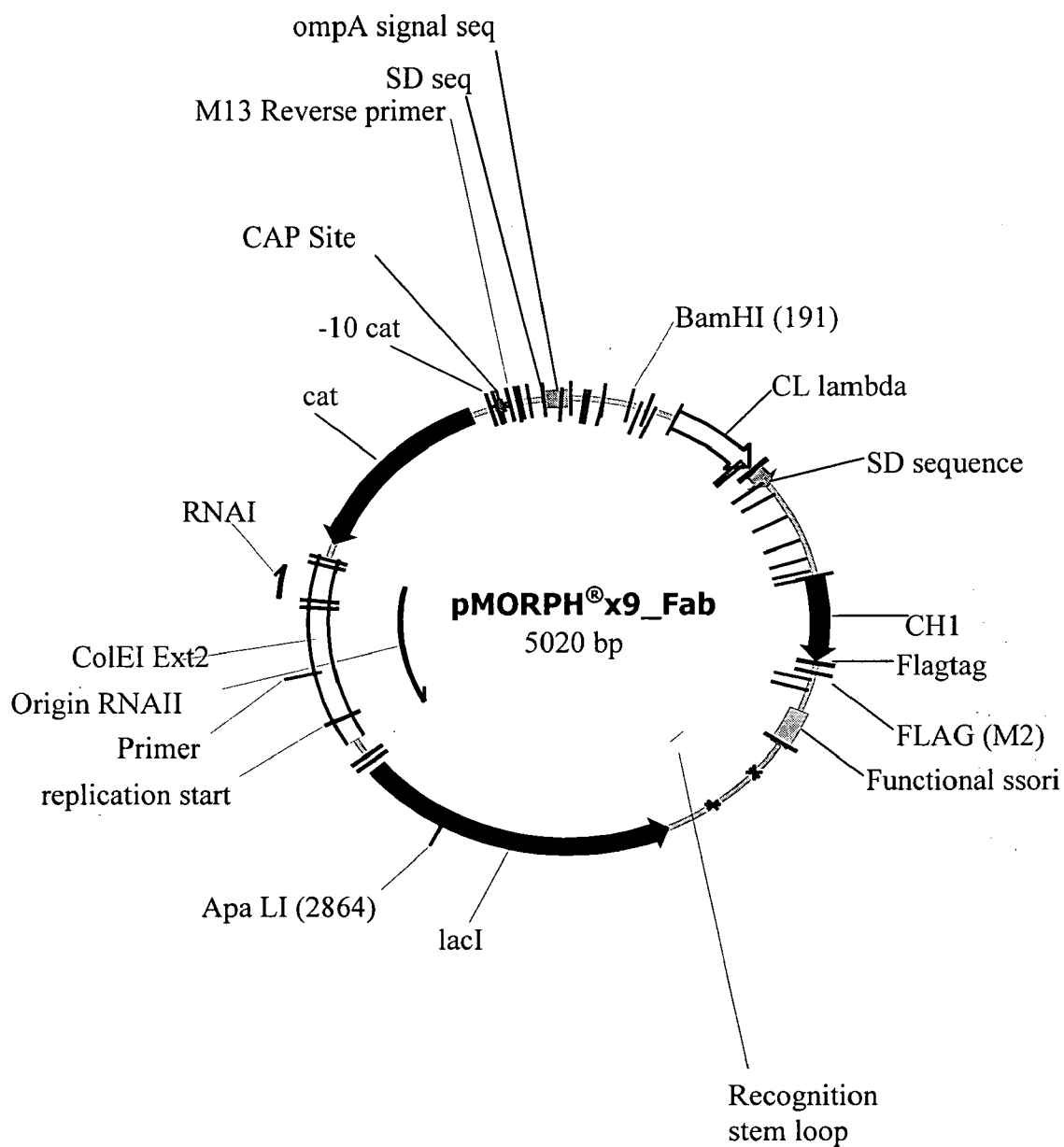


Figure 29

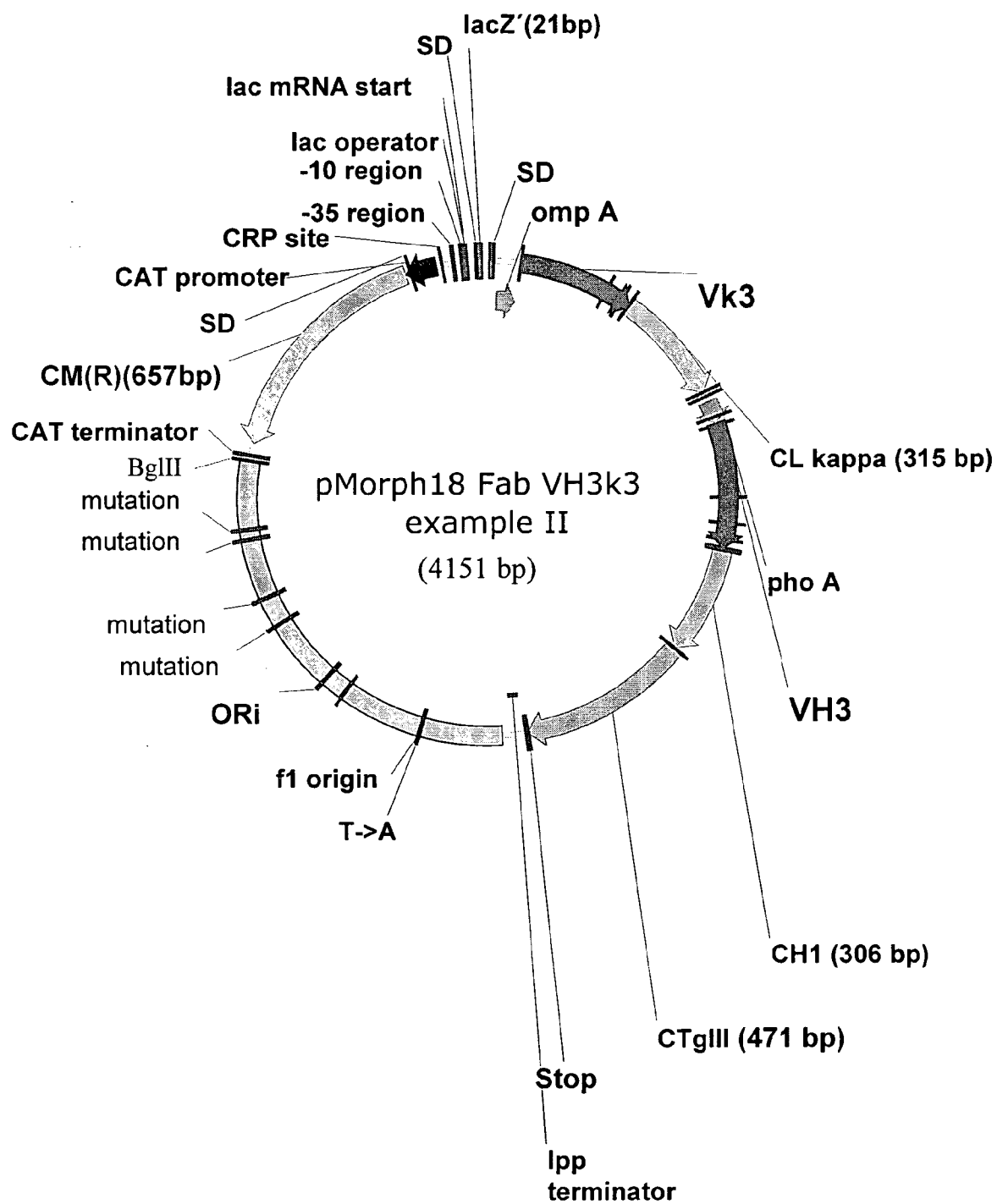


Figure 30

VL		Framework 1																	
Position		1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8
		EcoRV																	
		GAT	ATC	CAG	ATG	ACC	CAG	AGC	CCG	TCT	AGC	CTG	AGC	GCG	AGC	GTG	GGT	GAT	CGT
VLk1 (SEQ ID NO: 61)		GAT	ATC	CAG	ATG	ACC	CAG	AGC	CCG	TCT	AGC	CTG	AGC	GCG	AGC	GTG	GGT	GAT	CGT
MSPro28 (SEQ ID NO: 62)		GAT	ATC	CAG	ATG	ACC	CAG	AGC	CCG	TCT	AGC	CTG	AGC	GCG	AGC	GTG	GGT	GAT	CGT
VLk3 (SEQ ID NO: 63)		GAT	ATC	GTG	CTG	ACC	CAG	AGC	CCG	GCG	ACC	CTG	AGC	CTG	TCT	CCG	GGC	GAA	CGT
MSPro24 (SEQ ID NO: 64)		GAT	ATC	GTG	CTG	ACC	CAG	AGC	CCG	GCG	ACC	CTG	AGC	CTG	TCT	CCG	GGC	GAA	CGT
MSPro29 (SEQ ID NO: 65)		GAT	ATC	GTG	CTG	ACC	CAG	AGC	CCG	GCG	ACC	CTG	AGC	CTG	TCT	CCG	GGC	GAA	CGT
VLk4 (SEQ ID NO: 66)		GAT	ATC	GTG	ATG	ACC	CAG	AGC	CCG	GAT	AGC	CTG	GCG	GTG	AGC	CTG	GGC	GAA	CGT
MSPro21 (SEQ ID NO: 67)		GAT	ATC	GTG	ATG	ACC	CAG	AGC	CCG	GAT	AGC	CTG	GCG	GTG	AGC	CTG	GGC	GAA	CGT
VLl2 (SEQ ID NO: 68)		GAT	ATC	GCA	CTG	ACC	CAG	CCA	GCT		TCA	GTG	AGC	GGC	TCA	CCA	GGT	CAG	AGC
MSPro55 (SEQ ID NO: 69)		GAT	ATC	GCA	CTG	ACC	CAG	CCA	GCT		TCA	GTG	AGC	GGC	TCA	CCA	GGT	CAG	AGC
MSPro11 (SEQ ID NO: 70)		GAT	ATC	GCA	CTG	ACC	CAG	CCA	GCT		TCA	GTG	AGC	GGC	TCA	CCA	GGT	CAG	AGC
MSPro26 (SEQ ID NO: 71)		GAT	ATC	GCA	CTG	ACC	CAG	CCA	GCT		TCA	GTG	AGC	GGC	TCA	CCA	GGT	CAG	AGC
VLl3 (SEQ ID NO: 72)		GAT	ATC	GAA	CTG	ACC	CAG	CCG	CCT		TCA	GTG	AGC	GTT	GCA	CCA	GGT	CAG	ACC
MSPro54 (SEQ ID NO: 73)		GAT	ATC	GAA	CTG	ACC	CAG	CCG	CCT		TCA	GTG	AGC	GTT	GCA	CCA	GGT	CAG	ACC
MSPro2 (SEQ ID NO: 74)		GAT	ATC	GAA	CTG	ACC	CAG	CCG	CCT		TCA	GTG	AGC	GTT	GCA	CCA	GGT	CAG	ACC
MSPro12 (SEQ ID NO: 75)		GAT	ATC	GAA	CTG	ACC	CAG	CCG	CCT		TCA	GTG	AGC	GTT	GCA	CCA	GGT	CAG	ACC
MSPro59 (SEQ ID NO: 76)		GAT	ATC	GAA	CTG	ACC	CAG	CCG	CCT		TCA	GTG	AGC	GTT	GCA	CCA	GGT	CAG	ACC
VH		MfeI																	
		GAA/CAG	GTG	CAA	TTG	GTT	CAG	TCT	GGC	GCG	GAA	GTG	AAA	AAA	CCG	GGC	AGC	AGC	GTG
VH1A (SEQ ID NO: 77)		CAG	GTG	CAA	TTG	GTT	CAG	TCT	GGC	GCG	GAA	GTG	AAA	AAA	CCG	GGC	AGC	AGC	GTG
MSPro21 (SEQ ID NO: 78)		CAG	GTG	CAA	TTG	GTT	CAG	TCT	GGC	GCG	GAA	GTG	AAA	AAA	CCG	GGC	AGC	AGC	GTG
MSPro24 (SEQ ID NO: 79)		CAG	GTG	CAA	TTG	GTT	CAG	TCT	GGC	GCG	GAA	GTG	AAA	AAA	CCG	GGC	AGC	AGC	GTG
MSPro28 (SEQ ID NO: 80)		CAG	GTG	CAA	TTG	GTT	CAG	TCT	GGC	GCG	GAA	GTG	AAA	AAA	CCG	GGC	AGC	AGC	GTG
VH1B (SEQ ID NO: 81)		GAA/CAG	GTG	CAA	TTG	GTT	CAG	AGC	GGC	GCG	GAA	GTG	AAA	AAA	CCG	GGC	GCG	AGC	GTG
MSPro54 (SEQ ID NO: 82)		CAG	GTG	CAA	TTG	GTT	CAG	AGC	GGC	GCG	GAA	GTG	AAA	AAA	CCG	GGC	GCG	AGC	GTG
MSPro55 (SEQ ID NO: 83)		CAG	GTG	CAA	TTG	GTT	CAG	AGC	GGC	GCG	GAA	GTG	AAA	AAA	CCG	GGC	GCG	AGC	GTG
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MSPro11 (SEQ ID NO: 85)		CAG	GTG	CAA	TTG	GTT	CAG	AGC	GGC	GCG	GAA	GTG	AAA	AAA	CCG	GGC	GCG	AGC	GTG
MSPro26 (SEQ ID NO: 86)		CAG	GTG	CAA	TTG	GTT	CAG	AGC	GGC	GCG	GAA	GTG	AAA	AAA	CCG	GGC	GCG	AGC	GTG
MSPro29 (SEQ ID NO: 87)		CAG	GTG	CAA	TTG	GTT	CAG	AGC	GGC	GCG	GAA	GTG	AAA	AAA	CCG	GGC	GCG	AGC	GTG
VH2 (SEQ ID NO: 88)		GAA/CAG	GTG	CAA	TTG	AAA	GAA	AGC	GGC	CCG	GCC	CTG	GTG	AAA	CCG	ACC	CAA	ACC	CTG
MSPro12 (SEQ ID NO: 89)		CAG	GTG	CAA	TTG	AAA	GAA	AGC	GGC	CCG	GCC	CTG	GTG	AAA	CCG	ACC	CAA	ACC	CTG
VH6 (SEQ ID NO: 90)		GAA/CAG	GTG	CAA	TTG	CAA	CAG	TCT	GGT	CCG	GGC	CTG	GTG	AAA	CCG	AGC	CAA	ACC	CTG
MSPro59 (SEQ ID NO: 91)		CAG	GTG	CAA	TTG	CAA	CAG	TCT	GGT	CCG	GGC	CTG	GTG	AAA	CCG	AGC	CAA	ACC	CTG

Figure 30 cont.

Framework 4																
J	k	1	1	2	3	4	5	6	7	8	9	0	1	2	3	
BlpI																
StyI																
x	x	x	GAT	x	TGG	GSC	CAA	GSC	ACC	CTG	GTG	ACG	GTT	AGC	TCA	GC
CCT	TTT	TCT	GAT	GTT	TGG	GSC	CAA	GSC	ACC	CTG	GTG	ACG	GTT	AGC	TCA	GC
TAT	ACT	TTT	GAT	TAT	TGG	GSC	CAA	GSC	ACC	CTG	GTG	ACG	GTT	AGC	TCA	GC
TAT	CTT	TTT	GAT	CTT	TGG	GSC	CAA	GSC	ACC	CTG	GTG	ACG	GTT	AGC	TCA	GC
x	x	x	GAT	x	TGG	GSC	CAA	GSC	ACC	CTG	GTG	ACG	GTT	AGC	TCA	GC
CCT	CAT	TTT	GAT	TAT	TGG	GSC	CAA	GSC	ACC	CTG	GTG	ACG	GTT	AGC	TCA	GC
GTT	CTT	TTT	GAT	CAT	TGG	GSC	CAA	GSC	ACC	CTG	GTG	ACG	GTT	AGC	TCA	GC
TAT	GAG	TTT	GAT	TAT	TGG	GSC	CAA	GSC	ACC	CTG	GTG	ACG	GTT	AGC	TCA	GC
GGT	TTT	ATT	GAT	TAT	TGG	GSC	CAA	GSC	ACC	CTG	GTG	ACG	GTT	AGC	TCA	GC
GGT	TAT	TTT	GAT	AAT	TGG	GSC	CAA	GSC	ACC	CTG	GTG	ACG	GTT	AGC	TCA	GC
TAT	TAT	TTT	GAT	ATT	TGG	GSC	CAA	GSC	ACC	CTG	GTG	ACG	GTT	AGC	TCA	GC
x	x	x	GAT	x	TGG	GSC	CAA	GSC	ACC	CTG	GTG	ACG	GTT	AGC	TCA	GC
TAT	ATG	TTT	GAT	TAT	TGG	GSC	CAA	GSC	ACC	CTG	GTG	ACG	GTT	AGC	TCA	GC
x	x	x	GAT	x	TGG	GSC	CAA	GSC	ACC	CTG	GTG	ACG	GTT	AGC	TCA	GC
CCT	GAT	TTT	GAT	TAT	TGG	GSC	CAA	GSC	ACC	CTG	GTG	ACG	GTT	AGC	TCA	GC

**ANTIBODIES THAT BLOCK RECEPTOR
PROTEIN TYROSINE KINASE ACTIVATION,
METHODS OF SCREENING FOR AND USES
THEREOF**

**CROSS-REFERENCE TO RELATED
APPLICATIONS**

[0001] This application is a continuation of International Application No. PCT/IL02/00494 filed Jun. 20, 2002, the content of which is expressly incorporated herein by reference thereto, and which claims the benefit of U.S. Provisional Application No. 60/299,187 filed Jun. 20, 2001.

FIELD OF THE INVENTION

[0002] The present invention relates to immunoglobulins and functional fragments thereof useful for blocking activation of receptor protein tyrosine kinases, methods for screening for such immunoglobulins, compositions comprising said immunoglobulins and methods of using the same for treating or inhibiting disease, such as skeletal dysplasia, craniosynostosis disorders, cell proliferative diseases or disorders, or tumor progression.

BACKGROUND OF THE INVENTION

[0003] A wide variety of biological processes involves complex cellular communication mechanisms. One of the primary means of continual exchange of information between cells and their internal and external environments is via the secretion and specific binding of peptide growth factors. Growth factors exert pleiotropic effects and play important roles in oncogenesis and the development of multicellular organisms regulating cell growth, differentiation and migration. Many of these factors mediate their effects by binding to specific cell surface receptors. The ligand-activated receptors start an enzymatic signal transduction cascade from the cell membrane to the cell nucleus, resulting in specific gene regulation and diverse cellular responses.

[0004] Protein Kinases

[0005] One of the key biochemical mechanisms of signal transduction involves the reversible phosphorylation of proteins, which enables regulation of the activity of mature proteins by altering their structure and function.

[0006] Protein kinases ("PKs") are enzymes that catalyze the phosphorylation of hydroxy groups on tyrosine, serine and threonine residues of proteins. The consequences of this seemingly simple activity are staggering; cell growth, differentiation and proliferation; e.g., virtually all aspects of cell life in one way or another depend on PK activity. Furthermore, abnormal PK activity has been related to a host of disorders, ranging from relatively non-life threatening diseases such as psoriasis to extremely virulent diseases such as glioblastoma.

[0007] The kinases fall largely into two groups, those specific for phosphorylating serine and threonine, and those specific for phosphorylating tyrosine. Some kinases, referred to as "dual specificity" kinases, are able to phosphorylate tyrosine as well as serine/threonine residues.

[0008] Protein kinases can also be characterized by their location within the cell. Some kinases are transmembrane

receptor proteins capable of binding ligands external to the cell membrane. Binding the ligands alters the receptor protein kinase's catalytic activity. Others are non-receptor proteins lacking a transmembrane domain and yet others are ecto-kinases that have a catalytic domain on the extracellular (ecto) portion of a transmembrane protein or which are secreted as soluble extracellular proteins.

[0009] Many kinases are involved in regulatory cascades where their substrates may include other kinases whose activities are regulated by their phosphorylation state. Thus, activity of a downstream effector is modulated by phosphorylation resulting from activation of the pathway.

[0010] Receptor protein tyrosine kinases (RPTKs) are a subclass of transmembrane-spanning receptors endowed with intrinsic, ligand-stimulatable tyrosine kinase activity. RPTK activity is tightly controlled. When mutated or altered structurally, RPTKs can become potent oncoproteins, causing cellular transformation. In principle, for all RPTKs involved in cancer, oncogenic deregulation results from relief or perturbation of one or several of the auto-control mechanisms that ensure the normal repression of catalytic domains. More than half of the known RPTKs have been repeatedly found in either mutated or overexpressed forms associated with human malignancies (including sporadic cases; Blume-Jensen et al., 2001). RPTK overexpression leads to constitutive kinase activation by increasing the concentration of dimers. Examples are Neu/ErbB2 and epidermal growth factor receptor (EGFR), which are often amplified in breast and lung carcinomas and the fibroblast growth factors (FGFR) associated with skeletal and proliferative disorders (Blume-Jensen et al., 2001).

[0011] Fibroblast Growth Factors

[0012] Normal growth, as well as tissue repair and remodeling, require specific and delicate control of activating growth factors and their receptors. Fibroblast Growth Factors (FGFs) constitute a family of over twenty structurally related polypeptides that are developmentally regulated and expressed in a wide variety of tissues. FGFs stimulate proliferation, cell migration and differentiation and play a major role in skeletal and limb development, wound healing, tissue repair, hematopoiesis, angiogenesis, and tumorigenesis (reviewed in Ornitz and Itoh, 2001).

[0013] The biological action of FGFs is mediated by specific cell surface receptors belonging to the RPTK family of protein kinases. These proteins consist of an extracellular ligand binding domain, a single transmembrane domain and an intracellular tyrosine kinase domain which undergoes phosphorylation upon binding of FGF. The FGF receptor (FGFR) extracellular region contains three immunoglobulin-like (Ig-like) loops or domains (D1, D2 and D3), an acidic box, and a heparin binding domain. Five FGFR genes that encode for multiple receptor protein variants have been identified to date.

[0014] Another major class of cell surface binding sites includes binding sites for heparan sulfate proteoglycans (HSPG) that are required for high affinity interaction and activation of all members of the FGF family. Tissue-specific expression of heparan sulfate structural variants confer ligand-receptor specificity and activity of FGFs.

[0015] FGFR-Related Disease

[0016] Recent discoveries show that a growing number of skeletal abnormalities, including achondroplasia, the most common form of human dwarfism, result from mutations in FGFRs. Specific point mutations in different domains of FGFR3 are associated with autosomal dominant human skeletal disorders including hypochondroplasia, severe achondroplasia with developmental delay and acanthosis nigricans (SADDAN) and thanatophoric dysplasia (TD) (Cappellen et al., 1999; Webster et al., 1997; Tavormina et al., 1999). FGFR3 mutations have also been described in two craniosynostosis phenotypes: Muenke coronal craniosynostosis (Bellus et al., 1996; Muenke et al., 1997) and Crouzon syndrome with acanthosis nigricans (Meyers et al., 1995). Crouzon syndrome is associated with specific point mutations in FGFR2 and both familial and sporadic forms of Pfeiffer syndrome are associated with mutations in FGFR1 and FGFR2 (Galvin et al., 1996; Schell et al., 1995). Mutations in FGFRs result in constitutive activation of the mutated receptors and increased receptor protein tyrosine kinase activity, rendering cells and tissue unable to differentiate. Specifically, the achondroplasia mutation results in enhanced stability of the mutated receptor, dissociating receptor activation from down-regulation, leading to restrained chondrocyte maturation and bone growth inhibition (reviewed in Vajo et al., 2000).

[0017] There is accumulating evidence for mutations activating FGFR3 in various types of cancer. Constitutively activated FGFR3 in a large proportion of two common epithelial cancers, bladder and cervix, as well as in multiple myeloma, is the first evidence of an oncogenic role for FGFR3 in carcinomas. FGFR3 currently appears to be the most frequently mutated oncogene in bladder cancer where it is mutated in almost 50% of the cases and in about 70% of cases having recurrent superficial bladder tumors (Cappellen, et al, 1999; van Rhijn, et al, 2001; Billerey, et al, 2001). FGFR3 mutations are seen in 15-20% of multiple myeloma cases where point mutations that cause constitutive activation directly contribute to tumor development and progression (Chesi, et al, 1997; Plowright, et al, 2000, Ronchetti, et al, 2001).

[0018] In this context, the consequences of FGFR3 signaling appear to be cell type-specific. In chondrocytes, FGFR3 hyperactivation results in growth inhibition (reviewed in Ornitz, 2001), whereas in the myeloma cell it contributes to tumor progression (Chesi et al., 2001).

[0019] In view of the link between RPTK-related cellular activities and a number of human disorders various strategies have been employed to target the receptors and/or their variants for therapy. Some of these have involved biomimetic approaches using large molecules patterned on those involved in the cellular processes, e.g., mutant ligands (U.S. Pat. No. 4,966,849); soluble receptors and antibodies (WO 94/10202, U.S. Pat. No. 6,342,219); RNA ligands (U.S. Pat. No. 5,459,015) and tyrosine kinase inhibitors (WO 94/14808; U.S. Pat. No. 5,330,992).

[0020] Antibody Therapy

[0021] The search for new therapies to treat cancer and other diseases associated with growth factors and their corresponding cell surface receptors has resulted in the development of humanized antibodies capable of inhibiting

receptor function. For example, U.S. Pat. Nos. 5,942,602 and 6,365,157 disclose monoclonal antibodies specific for the HER2/neu and VEGF receptors, respectively. U.S. Pat. No. 5,840,301 discloses a chimeric, humanized monoclonal antibody that binds to the extracellular domain of VEGF and neutralizes ligand-dependent activation. U.S. Pat. No. 5,707,632 discloses a method for producing an antibody to a FGFR and a monoclonal antibody to FGFR that blocks binding of fibroblast growth factor to said fibroblast growth factor receptor sequences.

[0022] There remains an unmet need for highly selective molecules capable of blocking aberrant constitutive receptor protein tyrosine kinase activity, in particular FGFR activity, thereby addressing the clinical manifestations associated with the above-mentioned mutations, and modulating various biological functions.

[0023] Citation of any document herein is not intended as an admission that such document is pertinent prior art, or considered material to the patentability of any claim of the present application. Any statement as to content or a date of any document is based on the information available to applicant at the time of filing and does not constitute an admission as to the correctness of such a statement.

SUMMARY OF THE INVENTION

[0024] In one aspect the present invention provides molecules which are able to block receptor protein tyrosine kinase (RPTK) activity.

[0025] In another aspect the present invention provides molecules which are able to block fibroblast growth factor receptor (FGFR) activity, preferably fibroblast growth factor receptor 3 (FGFR3) activity.

[0026] In yet another aspect the present invention provides polypeptides encoding molecules which are able to block receptor protein tyrosine kinase (RPTK) activity, preferably FGFR activity, and more preferably FGFR3 activity.

[0027] In a further aspect the present invention provides a method to screen for molecules which are able to block receptor protein tyrosine kinase activity.

[0028] In one aspect the present invention provides a pharmaceutical composition comprising as an active ingredient a therapeutically effective amount of molecules of the invention useful in treating or preventing skeletal and proliferative diseases and disorders.

[0029] In another aspect the present invention provides a method for inhibiting growth of tumor cells associated with ligand-dependent or constitutive activation of a RPTK. According to one embodiment the RPTK is a fibroblast growth factor receptor. According to another embodiment the RPTK is FGFR3.

[0030] In yet a further aspect the present invention provides a method for treating skeletal disorders associated with ligand-dependent or constitutive activation of a RPTK. According to one embodiment the RPTK is a fibroblast growth factor receptor. According to another embodiment the RPTK is FGFR3.

[0031] In one aspect the present invention provides a method for blocking receptor protein tyrosine kinase acti-

vation in the cells of patients in need thereof by treatment with molecules capable of inhibiting receptor protein tyrosine kinase function.

[0032] In yet another aspect the present invention provides a method for inhibiting tumor growth, tumor progression or metastases.

[0033] In yet another aspect the present invention provides molecules useful for in vivo imaging of diseased states. In yet another aspect the present invention provides a kit comprising molecules of the invention. For example, a kit would comprise an antigen binding molecule of the invention and at least one reagent suitable for detecting the presence of said molecule when bound to said receptor protein tyrosine kinase and instructions for use.

[0034] These and other aspects are met by the invention disclosed herein.

[0035] The present invention provides a molecule comprising the antigen-binding portion of an antibody which has a specific affinity for a receptor protein tyrosine kinase (RPTK) and which blocks ligand-independent (constitutive activation) of a receptor protein tyrosine kinase. The present invention further provides a molecule that comprises the antigen-binding portion of an antibody which has a specific affinity for a receptor protein tyrosine kinase and which blocks ligand-dependent activation of a fibroblast growth factor receptor (FGFR), including FGFR1 and FGFR3.

[0036] Certain mutations in the genes of receptor protein tyrosine kinases result in activation of the receptor in a manner that is independent of the presence of a ligand. Such ligand-independent, or constitutive, receptor protein tyrosine kinase activation results in increased receptor activity. The clinical manifestations of certain mutations in fibroblast growth factor receptors (FGFR) are skeletal and proliferative disorders and diseases, including achondroplasia and various cancers.

[0037] Specific molecules of the present invention were found to inhibit or block constitutive activation of the FGFR3. Generation of inhibitory molecules would be useful for developing medicaments for use in treating or preventing skeletal and proliferative diseases and disorders associated with constitutive activation of receptor protein tyrosine kinases.

[0038] The present invention is directed to novel molecules comprising an antigen binding domain which binds to a receptor protein tyrosine kinase and blocks constitutive activation of said receptor protein tyrosine kinase. The molecules of the invention maybe antibodies or antigen binding fragments thereof.

[0039] One embodiment of the present invention provides a molecule which binds to the extracellular domain of a receptor protein tyrosine kinase and blocks constitutive and ligand-dependent activation of the receptor.

[0040] A currently more preferred embodiment of the present invention provides a molecule which binds to the extracellular domain of an FGF receptor and blocks constitutive and ligand-dependent activation of the receptor.

[0041] A currently most preferred embodiment of the present invention provides a molecule which binds FGFR3 and blocks constitutive and ligand-dependent activation of

the receptor, comprising V_L-CDR3 and V_H-CDR3 regions having amino acid SEQ ID NO:25 and SEQ ID NO:24, respectively and the corresponding isolated nucleic acid molecules comprising polynucleotide sequence SEQ ID NO:51 and SEQ ID NO:50.

[0042] A currently most preferred embodiment of the present invention provides a molecule which binds FGFR3 and blocks constitutive and ligand-dependent activation of the receptor, comprising V_L-CDR3 and V_H-CDR3 regions having SEQ ID NO:13 and SEQ ID NO:12 or SEQ ID NO:9 and SEQ ID NO:8, respectively and the corresponding isolated nucleic acid molecules comprising polynucleotide sequence SEQ ID NO:35 and SEQ ID NO:34 or SEQ ID NO:31 and SEQ ID NO:30.

[0043] Another currently preferred embodiment of the present invention provides a molecule herein denoted MSPRO12 comprising a variable light chain (V_L) having SEQ ID NO:94 and a variable heavy chain (V_H) having amino acid SEQ ID NO:105 and the corresponding isolated nucleic acid molecules comprising polynucleotide sequences having SEQ ID NO:75 and SEQ ID NO:89, respectively.

[0044] Another currently preferred embodiment of the present invention provides a molecule herein denoted MSPRO2 comprising a variable light chain (V_L) having SEQ ID NO:92 and a variable heavy chain (V_H) having SEQ ID NO:103 and the corresponding isolated nucleic acid molecules comprising polynucleotide sequences having SEQ ID NO:74 and SEQ ID NO:84.

[0045] A currently most preferred embodiment of the present invention provides a molecule, herein denoted MSPRO59, comprising a variable light chain (V_L) having SEQ ID NO:102 and a variable heavy chain (V_H) having SEQ ID NO:113 having the corresponding isolated nucleic acid molecules comprising polynucleotide sequences having SEQ ID NO:76 and SEQ ID NO:91, respectively.

[0046] According to the principles of the present invention, molecules which bind FGFR and block ligand-dependent receptor activation are provided. These molecules are useful in treating disorders and diseases associated with an FGFR that is activated in a ligand-dependent manner including certain skeletal disorders, hyperproliferative diseases or disorders and non-neoplastic angiogenic pathologic conditions such as neovascular glaucoma, macular degeneration, hemangiomas, angiofibromas, psoriasis and proliferative retinopathy including proliferative diabetic retinopathy.

[0047] In one embodiment the present invention provides a molecule which binds FGFR3 and blocks ligand-dependent activation of the receptor, comprising V_H-CDR3 and V_L-CDR3 regions having SEQ ID NO:20 and SEQ ID NO:21, respectively and the corresponding polynucleotide sequence having SEQ ID NO:44 and SEQ ID NO:45, respectively. In another embodiment the present invention provides a molecule comprising a variable light chain (V_L) having SEQ ID NO:99 and a variable heavy chain (V_H) having SEQ ID NO:110, having the corresponding isolated nucleic acid molecules comprising polynucleotide sequences having SEQ ID NO:65 and SEQ ID NO:87, respectively.

[0048] Other embodiments of the present invention provide a molecule which binds FGFR3 and blocks ligand-

dependent activation of the receptor, comprising V_H-CDR3 and V_L-CDR3 regions selected from SEQ ID NO:10 and SEQ ID NO:11; SEQ ID NO:14 and SEQ ID NO:15; SEQ ID NO:16 and SEQ ID NO:17; SEQ ID NO:18 and SEQ ID NO:19; SEQ ID NO:26 and SEQ ID NO:27 and SEQ ID NO:28 and SEQ ID NO:29 and the corresponding isolated nucleic acid molecules comprising polynucleotide sequences having SEQ ID NO according to Table 1B.

[0049] Additional embodiments of the present invention provide molecules having an antigen binding domain comprising a VL region and a VH region, respectively, selected from SEQ ID NO:93 and SEQ ID NO:104; SEQ ID NO:95 and SEQ ID NO:106; SEQ ID NO:96 and SEQ ID NO:107; SEQ ID NO:97 and SEQ ID NO:108; SEQ ID NO:98 and SEQ ID NO:109; SEQ ID NO:99 and SEQ ID NO:110; and SEQ ID NO:101 and SEQ ID NO:112 and the corresponding isolated nucleic acid molecules comprising polynucleotide sequences having SEQ ID NO:70 and SEQ ID NO:85; SEQ ID NO:67 and SEQ ID NO:78; SEQ ID NO:64 and SEQ ID NO:79; SEQ ID NO:71 and SEQ ID NO:86; SEQ ID NO:62 and SEQ ID NO:80; SEQ ID NO:65 and SEQ ID NO:87; and SEQ ID NO:69 and SEQ ID NO:83.

[0050] One embodiment of the present invention provides a molecule comprising V_H-CDR3 and V_L-CDR3 domains of amino acid sequences having SEQ ID NO:22 and SEQ ID NO:23, which has specific affinity for FGFR1 and which blocks ligand-dependent activation of FGFR1, and the corresponding polynucleotide sequences having SEQ ID NO:46 and SEQ ID NO:47.

[0051] Another embodiment of the present invention provides a molecule comprising V_H and V_L domains of amino acid sequences having SEQ ID NO:111 and 100, which has specific affinity for FGFR1 and which blocks ligand-dependent activation of FGFR1, and the corresponding isolated nucleic acid molecules comprising polynucleotide sequences having SEQ ID NO:82 and SEQ ID NO:73.

[0052] The present invention also relates to methods for screening for the molecules according to the present invention by using a dimeric form of a receptor protein tyrosine kinase as a target for screening a library of antibody fragments.

[0053] According to one currently preferred embodiment the screening method comprises

[0054] providing a library of of antigen binding fragments;

[0055] screening said library for binding to a dimeric form of a receptor protein tyrosine kinase;

[0056] identifying an antigen binding fragment which binds to the dimeric form of the receptor protein tyrosine kinase as a candidate molecule for blocking constitutive activation of the receptor protein tyrosine kinase; and

[0057] determining whether the candidate molecule blocks constitutive and or ligand-dependent activation of the receptor protein tyrosine kinase in a cell.

[0058] According to another embodiment, the dimeric form of the RPTK is a soluble extracellular domain of a receptor protein tyrosine kinase. Non-limiting examples of receptor protein tyrosine kinases disclosed in Blume-Jensen

et al. (2001) include EGFR/ErbB1, ErbB2/HER2/Neu, ErbB/HER3, ErbB4/HER4, IGF-1R, PDGFR- α , PDGFR- β , CSF-1R, kit/SCFR, Flk2/FH3, Flk1/VEGFR1, Flk1/VEGFR2, Flt4/VEGFR3, FGFR1, FGFR2/K-SAM, FGFR3, FGFR4, TrkA, TrkC, HGFR, RON, EphA2, EphB2, EphB4, Axl, TIE/TIE1, Tek/TIE2, Ret, ROS, Alk, Ryk, DDR, LTK and MUSK. Heterodimeric form of the receptors may be used as antigen.

[0059] By using a dimeric form of the RPTK as bait in the screen, a molecule which would bind to the dimeric form of the receptor has been identified. This presents a novel concept in screening for antibodies or fragments thereof with the capacity to bind to a constitutively activated RPTK such as those associated with various disorders and diseases. It also presents an opportunity to screen for molecules which bind to a heterodimer RPTK. A further aspect of the present invention provides a pharmaceutical composition comprising as an active ingredient a therapeutically effective amount of a molecule of the present invention in a pharmaceutically acceptable carrier or excipient useful for preventing or treating skeletal or cartilage diseases or disorders and craniosynostosis disorders associated with constitutive or ligand-dependent activation of a receptor protein tyrosine kinase.

[0060] In one embodiment the pharmaceutical compositions of the present invention may be used for treating or preventing skeletal disorders associated with aberrant FGFR signaling, including achondroplasia, thanatophoric dysplasia, Apert or Pfeiffer syndrome and related craniosynostosis disorders.

[0061] A further aspect of the present invention provides a pharmaceutical composition comprising as an active ingredient a therapeutically effective amount of a molecule of the present invention in a pharmaceutically acceptable carrier or excipient useful for preventing or treating cell proliferative diseases or disorders or tumor progression, associated with the constitutive or ligand-dependent activation of a receptor protein tyrosine kinase.

[0062] In one embodiment the pharmaceutical compositions of the present invention may be used for treating or preventing proliferative diseases associated with aberrant FGFR signaling, including multiple myeloma, transitional cell carcinoma of the bladder, mammary and cervical carcinoma, chronic myeloid leukemia and osteo- or chondrosarcoma.

[0063] A further aspect of the invention provides molecules comprising an antigen binding domain which can be conjugated to a cytotoxin useful for targeting cells expressing said antigen. Another aspect of the present invention provides molecules comprising an antigen binding domain which can be conjugated to appropriate detectable imaging moiety, useful for in vivo tumor imaging.

[0064] A still further aspect of the present invention provides methods for treating or inhibiting the aforementioned diseases and disorders by administering a therapeutically effective amount of a pharmaceutical composition comprising a molecule of the present invention to a subject in need thereof.

[0065] Other aspects of the invention will be apparent upon consideration of the following description, figures and embodiments.

BRIEF DESCRIPTION OF THE DRAWINGS

[0066] **FIG. 1** shows hFR3²³⁻³⁷⁴TDhis purification by Coomassie stained SDS-PAGE.

[0067] **FIG. 2** shows hFR3²³⁻³⁷⁴TDhis binding to heparin and FGF9.

[0068] **FIG. 3** shows the purification of FR3exFc and FR1exFc on SDS-PAGE.

[0069] **FIG. 4** shows the neutralization effect of the hFR3²³⁻³⁷⁴TDhis and FR3exFc soluble receptors in a ligand-dependent proliferation assay.

[0070] **FIG. 5** shows the effect of MS-PRO Fabs on proliferation of FGFR-expressing cells.

[0071] **FIG. 6** shows the effect of MSPRO Fabs on proliferation of FGFR3-expressing cells.

[0072] **FIGS. 7A and 7B** show the neutralizing activity of several MSPRO Fabs in a proliferation assay using the FDCP-FR3 (C10; **FIG. 7A**) or the FDCP-FR1 cells (**FIG. 7B**).

[0073] **FIG. 8** shows the receptor specificity of MSPRO Fabs on RCJ cells expressing R3, ach, R1 or R3 receptors by Western blot using an anti-P-JNK (phosphorylated/activated Jun kinase) antibody. **FIG. 8A** shows different MSPRO Fabs while **FIG. 8B** shows a dose response of MSPRO 12, 29 and 13 on RCJ-FR3 cells.

[0074] **FIGS. 9A-9D** demonstrates the specificity and potency of MS-PRO Fabs by Western blot with anti-P-ERK (phosphorylated/activated ERK) antibody.

[0075] **FIG. 10** shows a diagrammatic representation of FGFR3 and of FGFR3 truncations (D2-3, D2) and isoforms (IIIb, IIIc). The isoform IIIb differs from IIIc at the carboxy terminus of the IgIII domain as indicated with a dotted line.

[0076] **FIG. 11** shows that the FGFR3 neutralizing Fabs recognize IgII or IgII and III in the extracellular region of FGFR3.

[0077] **FIG. 12** shows the proliferation level of FGFR3IIIb and FGFR3IIIc expressing cells in the presence of MSPRO29. MSPRO 29 specifically recognizes the IIIc isoform of FGFR3.

[0078] **FIGS. 13A and 13B** show the results of a proliferation assay for FDCP-FR3IIIb or FDCP-FR3IIIc cells incubated with increasing doses of the indicated Fabs.

[0079] **FIG. 14** shows iodinated MSPRO29 binding to FGFR3.

[0080] **FIG. 15** shows results of a proliferation assay is a graph wherein iodinated MSPRO29 retained its activity against FGFR3.

[0081] **FIGS. 16A-16F** show the selective binding of radiolabelled MSPRO29 to histological sections of growth plate.

[0082] **FIGS. 17A and 17B** show a proliferation assay of FDCP-FR3 (17A) and FDCP-FR3ach cells (17B) incubated with FGF9 and with increasing doses of indicated Fabs.

[0083] **FIG. 18B** shows that MSPRO12 and MSPRO59 inhibit the ligand independent proliferation of FDCP-

FR3ach cells. **FIG. 18A** shows analysis of the ligand-dependent FDCP-FR3 wt cells.

[0084] **FIG. 19** shows the restoration of cell growth by MS-PRO54 and MSPRO59.

[0085] **FIG. 20** represents the growth rate of treated bone with MS-PRO 59.

[0086] **FIG. 21** is a flow chart of the experimental protocol for assessing receptor activation and signaling.

[0087] **FIG. 22** shows ¹²⁵I-MSPRO59 localization to the FDCP-FR3ach derived tumor.

[0088] **FIG. 23** shows the effect of MSPRO59 on inhibiting ligand-independent tumor growth.

[0089] **FIG. 24** shows the effect of MSPRO59 on inhibiting ligand-independent tumor growth.

[0090] **FIG. 25A** shows the effect of MSPRO59 on inhibiting ligand-independent tumor growth. **FIG. 25B** shows scFv MSPRO59 blocking the proliferation of FDCP-FR3 (S375C) cells.

[0091] **FIG. 26** shows the effect of MSPRO59 single chain antibody on inhibiting ligand-independent tumor growth.

[0092] **FIG. 27** shows binding of Fab Miniantibodies to FGFR3-Fc and FGFR1-Fc (ELISA).

[0093] **FIG. 28** is an example of a Fab expression vector, having SEQ ID NO:53, for use in accordance with the present invention.

[0094] **FIG. 29** is an example of a phage display vector, having SEQ ID NO:54, for use in accordance with the present invention.

[0095] **FIG. 30** depicts the polynucleotide sequences of the VL and VH of MSPRO antibodies of the present invention SEQ ID NOS: 61-91.

DETAILED DESCRIPTION OF THE INVENTION

[0096] The present invention is based on the discovery that neutralizing antibodies that block ligand-dependent and ligand-independent activation of fibroblast growth factor receptor 3 (FGFR3), a receptor protein tyrosine kinase (RPTK), can be obtained by screening an antibody library against a dimeric form of the extracellular portion of FGFR3. Until the present invention, the present inventors are unaware of any success in obtaining neutralizing antibodies that block constitutive activation of any RPTK including FGFR or ligand-dependent FGFR activation.

[0097] For convenience certain terms employed in the specification, examples and claims are described herein.

[0098] The term "receptor protein tyrosine kinase" or "RPTK" as used herein and in the claims refers to a subclass of transmembrane-spanning receptors endowed with intrinsic, ligand-stimulatable tyrosine kinase activity. RPTKs comprise a large family of spatially and temporally regulated proteins that control many different aspects of growth and development. When mutated or altered structurally, RPTKs can undergo deregulation and become activated in a ligand-independent, or constitutive, manner. In certain cases they become potent oncoproteins, causing cellular transformation.

[0099] As used herein and in the claims the term “fibroblast growth factor receptor” or “FGFR” denotes a receptor specific for FGF which is necessary for transducing the signal exerted by FGF to the cell interior, typically comprising an extracellular ligand-binding domain, a single transmembrane helix, and a cytoplasmic domain having tyrosine kinase activity. The FGFR extracellular domain consists of three immunoglobulin-like (Ig-like) domains (D1, D2 and D3), a heparin binding domain and an acidic box. Alternative splicing of the FGF receptor mRNAs generates different variants of the receptors. Certain abbreviations are employed herein including “FR3” for FGFR3 and “FR1” for FGFR1.

[0100] Molecules, including antibodies and fragments thereof, comprising an antigen binding domain to a receptor protein tyrosine kinase are highly necessary for the treatment of various pathological conditions.

[0101] In the past, the laboratory of the present inventors encountered difficulties in raising neutralizing antibodies against FGFR3. When mice were immunized with the soluble monomeric FGFR3 receptor, by the time the antibody titers began to increase, the mice died. The experiments performed in the laboratory of the present inventors that failed to obtain anti-FGFR3 neutralizing antibodies in mice are presented in the Examples. By using a soluble dimeric form of the extracellular domain of the FGFR3 receptor to screen for antibodies, e.g., Fabs, that bind from a phage display antibody library, the present inventors were able to overcome a problem in the prior art for which there was yet no solution and to obtain numerous high affinity ($K_D < 10$ nM) antibodies (Fabs) that bind FGFR3 and interfere with ligand binding, thereby blocking ligand-dependent activation of FGFR3 signaling. Very surprisingly, from among the group of Fabs that block ligand-dependent activation, Fab antibodies which also block ligand-independent (constitutive) activation of FGFR3 by blocking signaling caused by constitutive dimerization of FGFR3 were identified. To the best of the present inventors' knowledge, these Fab antibodies, which block constitutive activation of

FGFR3, are the first antibodies against any receptor protein tyrosine kinase that block constitutive, ligand-independent activation/signaling.

[0102] Trastuzumab, an anti-human epidermal growth factor receptor 2 (HER2) antibody, was the first humanized monoclonal antibody approved for therapeutic use. Its mode of action appears to be manifold, including HER2 down regulation, prevention of heterodimer formation, prevention of HER2 cleavage and others (Baselga and Albanell, 2001). U.S. Pat. No. 5,677,171; U.S. Pat. No. 5,772,997; U.S. Pat. No. 6,165,464; and U.S. Pat. No. 6,399,063 disclose the anti-HER2 invention but neither teach nor suggest that the antibody blocks ligand-independent receptor activation.

Embodiments of the Invention

[0103] One aspect of the present invention is directed to neutralizing antibodies and more generally to a molecule that comprises the antigen-binding portion of an antibody which blocks ligand-dependent activation and constitutive, ligand-independent activation of a receptor protein tyrosine kinase. According to one embodiment the RPTK is a fibroblast growth factor receptor. According to another embodiment the RPTK is FGFR3.

[0104] Another aspect of the present invention is directed to molecules comprising an antigen binding domain which blocks ligand-dependent activation of an FGFR. In one aspect the FGFR is FGFR3.

[0105] The molecule having the antigen-binding portion of an antibody according to the present invention can be used in a method for blocking the ligand-dependent activation and/or ligand independent (constitutive) activation of FGFR3. Preferred embodiments of such antibodies/molecules, obtained from an antibody library designated as HuCAL® (Human Combinatorial Antibody Library) clone, is presented in Table 1A with the unique V_H -CDR3 and V_L -CDR3 sequences given.

[0106] In addition to sequencing of the clones, a series of biochemical assays were performed to determine affinity and specificity of the molecules to the respective receptors.

TABLE 1A

HuCAL® - Clone	VH-CDR3 Sequence	VL-CDR3 Sequence	Framework
MSPRO2	DFLGYEFDY (SEQ ID NO: 8)	QSYDYSADY (SEQ ID NO: 9)	VH1B_L3
MSPRO11	YYGSSLYHYV FGGFIDY (SEQ ID NO: 10)	QSHHFYE (SEQ ID NO: 11)	VH1B_L2
MSPRO12	YHSWYEMGYG GSTVGYMFDY (SEQ ID NO: 12)	QSYDFDEA (SEQ ID NO: 13)	VH2_L3
MSPRO21	DNWFKPFSVDV (SEQ ID NO: 14)	QQYDSIPY (SEQ ID NO: 15)	VH1A_k4
MSPRO24	VNHWTYTFDY (SEQ ID NO: 16)	QQMSNYPD (SEQ ID NO: 17)	VH1A_k3
MSPRO26	GYWYAYFTYI NYGYFDN (SEQ ID NO: 18)	QSYDNNSDV (SEQ ID NO: 19)	VH1B_L2
MSPRO28	GGGWVSHGYG YLFDL (SEQ ID NO: 26)	FQYGSIPP (SEQ ID NO: 27)	VH1A_k1
MSPRO29	TWQYSYFYLL DGGYYFDI (SEQ ID NO: 20)	QQTNNAPV (SEQ ID NO: 21)	VH1B_k3
MSPRO54	NMAYTNYQYV NMPHFDY (SEQ ID NO: 22)	QSYDYFKL (SEQ ID NO: 23)	VH1B_L3
MSPRO55	SMNSTMYWYL RRVLFDH (SEQ ID NO: 28)	QSYDMYMYI (SEQ ID NO: 29)	VH1B_L2

TABLE 1A-continued

HuCAL ® - Clone	VH-CDR3 Sequence	VL-CDR3 Sequence Framework
MSPRO59	SYYPDFDY (SEQ ID NO: 24)	QSYDGPDLW (SEQ ID NO: 25)

VH refers to the variable heavy chain,
VL refers to the variable light chain;
L refers to the lambda light chain and
k refers to the kappa light chain.

[0107] Table 1B lists the corresponding polynucleotide sequences of the CDR domains.

TABLE 1B

HuCAL ® Clone	VH-CDR3 polynucleotide sequence	VL-CDR3 polynucleotide sequence
MSPRO2	GATTTTCTTG GTTATGAGTT TGATTAT (SEQ ID NO: 30)	CAGAGCTATG ACTATTCTGC TGATTAT (SEQ ID NO: 31)
MSPRO11	TATTATGGTT CTTCCTTTA TCATTATGTT TTTGGTGGTT TTATTGATTA T (SEQ ID NO: 32)	CAGTCTCATC ATTTTATGA G (SEQ ID NO: 33)
MSPRO12	TATCATTCCTT GGTATGAGAT GGGTATATAT GGTCTACTG TTGGTTATAT GTTGATTAT (SEQ ID NO: 34)	CAGAGCTATG ACTTTGATTT TGCT (SEQ ID NO: 35)
MSPRO21	GATAATTGGT TTAAGCCTTT TTCTGATGTT (SEQ ID NO: 36)	CAGCAGTATGAT TCTATTCCT TAT (SEQ ID NO: 37)
MSPRO24	GTTAATCATT GGACTTATAC TTTTGATTAT (SEQ ID NO: 38)	CAGCAGATGT CTAATTATCC TGAT (SEQ ID NO: 39)
MSPRO26	GGTTATTGGT ATGCTTATTT TACTTATATT AATTATGGTT ATTTTGATAA T (SEQ ID NO: 40)	CAGAGCTATG ACAATAATTC TGATGTT (SEQ ID NO: 41)
MSPRO28	GGTGGTGGTT GGGTTTCTCA TGGTTATAT TATCTTTTGG ATCTT (SEQ ID NO: 42)	TTTCAGTATG GTTCATTCC TCCT (SEQ ID NO: 43)
MSPRO29	ACTTGGCAGT ATTCTTATTT TTATTATCTT GATGGTGGTT ATTATTTTGA TATT (SEQ ID NO: 44)	CAGCAGACTA ATAATGCTCC TGTT (SEQ ID NO: 45)
MSPRO54	AATATGGCTT ATACTAATTA TCAGTATGTT AATATGCCTC ATTTTGATTA T (SEQ ID NO: 46)	CAGAGCTATG ACTATTTTAA GCTT (SEQ ID NO: 47)
MSPRO55	TCTATGAATT CTACTATGTA TTGGTAT CTTCGTCGTG TTCCTTTTGA TCAT (SEQ ID NO: 48)	CAGAGCTATGAC ATGTATAATTAT ATT (SEQ ID NO: 49)
MSPRO59	TCTTATTATC CTGATTTTGA TTAT (SEQ ID NO: 50)	CAGAGCTATGAC GGTCCTGATCTT TGG (SEQ ID NO: 51)

[0108] The polypeptide sequence of the VH and VL domains of the currently preferred embodiments of the

present invention are presented below. **FIG. 30** provides the polynucleotide sequences of the preferred embodiments of the invention.

MS-Pro-2-VL (SEQ ID NO: 92)	
1	DIELTQPPSV SVAPGQTARI SCSGDALGDK YASWYQQKPG QAPVLVIYDD
51	SDRPSGIPER FSGNSNGNTA TLTISGTQAE DEADYYCQSY DYSADYVFGG
101	GTKLTVLGQ
MS-Pro-11-VL (SEQ ID NO: 93)	
1	DIALTQPASV SGSPGQSITI SCTGTSSDVG GYNVSWYQQ HPGKAPKLM
51	YDVSNRPSGV SNRFSGSKSG NTASLTISGL QAEDADYYC QSHHFYEVFG
101	GGTKLTVLGQ
MS-PRO-12-VL (SEQ ID NO: 94)	
1	DIELTQPPSV SVAPGQTARI SCSGDALGDK YASWYQQKPG QAPVLVIYDD
51	SDRPSGIPER FSGNSNGNTA TLTISGTQAE DEADYYCQSY DFDFAVFGG
101	TKLTVLGQ

[0109] corresponding to polynucleotide sequence having SEQ ID NO:74

[0110] corresponding to polynucleotide sequence having SEQ ID NO:70

[0111] corresponding to polynucleotide sequence having SEQ ID NO:77

-continued

MS-Pro-21-VL (SEQ ID NO: 95)	
1	DIVMTQSPDS LAVSLGERAT INCRSSQSVL YSSNNKNYLA WYQQKPGQPP
51	KLLIWASTR ESGVPDRFSG SGSGTDFTLT ISSLQAEDVA VYYCQQYDSI
101	PYTFGQGTKV EIKRT

[0112] corresponding to polynucleotide sequence having
SEQ ID NO:67

MS-Pro-24-VL (SEQ ID NO: 96)	
1	DIVLTQSPAT LSLSPGERAT LSCRASQSVS SSYLAWYQQK PGQAPRLLIY
51	GASSRATGVP ARFSGSGSGT DFTLTISSE PEDFATYYCQ QMSNYPDTFG
101	QGTKVEIKRT

[0113] corresponding to polynucleotide sequence having
SEQ ID NO:64

MS-Pro-26-VL (SEQ ID NO: 97)	
1	DIALTQPASV SGSPGQSITI SCTGTSSDVG GYNYVSWYQQ HPGKAPKLMI
51	YDVSNRPSGV SNRFGSGKSG NTASLTISGL QAEDADYYC QSYDNNSDVV
101	FGGGTKLTVL GQ

[0114] corresponding to polynucleotide sequence having
SEQ ID NO:71

MS-Pro-28-VL (SEQ ID NO: 98)	
1	DIQMTQSPSS LSASVGDRVT ITCRASQGIS SYLAWYQQK GKAPKLLIYA
51	ASSLQSGVPS RFSGSGSGTD FTLTISLQP EDFAVYYCFQ YGSIPPTFGQ
101	GTKVEIKRT

[0115] corresponding to polynucleotide sequence having
SEQ ID NO:62

MS-Pro-29-VL (SEQ ID NO: 99)	
1	DIVLTQSPAT LSLSPGERAT LSCRASQSVS SSYLAWYQQK PGQAPRLLIY

MS-Pro-29-VL (SEQ ID NO: 99)	
51	GASSRATGVP ARFSGSGSGT DFTLTISSE PEDFATYYCQ QTNNAPVTFG
101	QGTKVEIKRT

[0116] corresponding to polynucleotide sequence having
SEQ ID NO:65

MS-Pro-54-VL (SEQ ID NO: 100)	
1	DIELTQPPSV SVAPGQTARI SCSGDALGDK YASWYQQKPG QAPVLVIYDD
51	SDRPSGIPER FSGSNSGNTA TLTISGTQAE DEADYYCQSY DYFKLVFGGG
101	TKLTVLGQ

[0117] corresponding to polynucleotide sequence having
SEQ ID NO:73

MS-Pro-55-VL (SEQ ID NO: 101)	
1	DIALTQPASV SGSPGQSITI SCTGTSSDVG GYNYVSWYQQ HPGKAPKLMI
51	YDVSNRPSGV SNRFGSGKSG NTASLTISGL QAEDADYYC QSYDMYNYIV
101	FGGGTKLTVL GQ

[0118] corresponding to polynucleotide sequence having
SEQ ID NO:69

MS-Pro-59-VL (SEQ ID NO: 102)	
1	DIELTQPPSV SVAPGQTARI SCSGDALGDK YASWYQQKPG QAPVLVIYDD
51	SDRPSGIPER FSGSNSGNTA TLTISGTQAE DEADYYCQSY DGPDLWVFGG
101	GTKLTVLGQ

[0119] corresponding to polynucleotide sequence having
SEQ ID NO:76

MS-Pro-2-VH (SEQ ID NO: 103)	
1	QVQLVQSGAE VKKPGASVKV SCKASGYTFT SYMHWVRQA PGQGLEWMGW

-continued

MS-Pro-2-VH (SEQ ID NO: 103)	
51	INPNSGGTNY AQKFQGRVTM TRDTSISTAY MELSSLRSED TAVYYCARDF
101	LGYEFDYWGQ GTLTVTVSS

[0120] corresponding to polynucleotide sequence having
SEQ ID NO:84

MS-Pro-11-VH (SEQ ID NO: 104)	
1	QVQLVQSGAE VKKPGASVKV SCKASGYTFT SYMHWVRQA PGQGLEWMGW
51	INPNSGGTNY AQKFQGRVTM TRDTSISTAY MELSSLRSED TAVYYCARY
101	GSSLYHYVFG GFIDYWGQGT LTVTVSS

[0121] corresponding to polynucleotide sequence having
SEQ ID NO:85

MS-Pro-12-VH (SEQ ID NO: 105)	
1	QVQLKESGPA LVKPTQTTLT TCTFSGFSLT TSGVGVGWIR QPPGKALEWL
51	ALIDWDDDKY YSTSLKTRLT ISKDTSKNQV VLTMTNMDPV DTATYICARY
101	HSWYEMGYYG STVGVMFDYW GQGLTVTVSS

[0122] corresponding to polynucleotide sequence having
SEQ ID NO:89

MS-Pro-21-VH (SEQ ID NO: 106)	
1	QVQLVQSGAE VKKPGSSVKV SCKASGGTFS SYAISWVRQA PGQGLEWMGG
51	IIPIFGTANY AQKFQGRVTI TADESTSTAY MELSSLRSED TAVYYCARDN
101	WFKPFSVDWG QGTLTVTVSS

[0123] corresponding to polynucleotide sequence having
SEQ ID NO:78

MS-Pro-24-VH (SEQ ID NO: 107)	
1	QVQLVQSGAE VKKPGSSVKV SCKASGGTFS SYAISWVRQA PGQGLEWMGG

-continued

MS-Pro-24-VH (SEQ ID NO: 107)	
51	IIPIFGTANY AQKFQGRVTI TADESTSTAY MELSSLRSED TAVYYCARVN
101	HWTYTFDYWG QGTLTVTVSS

[0124] corresponding to polynucleotide sequence having
SEQ ID NO:79

MS-Pro-26-VH (SEQ ID NO: 108)	
1	QVQLVQSGAE VKKPGASVKV SCKASGYTFT SYMHWVRQA PGQGLEWMGW
51	INPNSGGTNY AQKFQGRVTM TRDTSISTAY MELSSLRSED TAVYYCARGY
101	WYAYFTYINY GYFDNWGQGT LTVTVSS

[0125] corresponding to polynucleotide sequence having
SEQ ID NO:86

MS-Pro-28-VH (SEQ ID NO: 109)	
1	QVQLVQSGAE VKKPGSSVKV SCKASGGTFS SYAISWVRQA PGQGLEWMGG
51	IIPIFGTANY AQKFQGRVTI TADESTSTAY MELSSLRSED TAVYYCARGG
101	GWVSHGYIYL FDLWGQGLTV TVSS

[0126] corresponding to polynucleotide sequence having
SEQ ID NO:80

MS-Pro-29-VH (SEQ ID NO: 110)	
1	QVQLVQSGAE VKKPGASVKV SCKASGYTFT SYMHWVRQA PGQGLEWMGW
51	INPNSGGTNY AQKFQGRVTM TRDTSISTAY MELSSLRSED TAVYYCARTW
101	QYSYFYLDG GYFIDIWGQG TLTVTVSS

[0127] corresponding to polynucleotide sequence having
SEQ ID NO:87

MS-Pro-54-VH (SEQ ID NO: 111)	
1	QVQLVQSGAE VKKPGASVKV SCKASGYTFT SYMHWVRQA PGQGLEWMGW

-continued

MS-Pro-54-VH (SEQ ID NO: 111)	
51	INPNSGGTNY A QKFQGRVTM TRDTSISTAY MELSSLRSED TAVYYCARNM
101	AYTNYQYVNM PHFDYWGQGT LVTVSS

[0128] corresponding to polynucleotide sequence having SEQ ID NO:82

MS-Pro-55-VH (SEQ ID NO: 112)	
1	QVQLVQSGAE VKKPGASVKV SCKASGYTFT SYMHWVRQA PGQGLEWMGW
51	INPNSGGTNY A QKFQGRVTM TRDTSISTAY MELSSLRSED TAVYYCARSM
101	NSTMWYLLRR VLFHDHWGQGT LVTVSS

[0129] corresponding to polynucleotide sequence having SEQ ID NO:83

-continued

MS-Pro-59-VH (SEQ ID NO: 113)	
51	GRTYYRSKWY NDYAVSVKSR ITINPDTSKN QFSLQLNSVT PEDTAVYYCA
101	RSYYPDFDYW GQGT LVTVSS

[0130] corresponding to polynucleotide sequence having SEQ ID NO:91

[0131] In addition to sequencing of the clones, a series of biochemical assays were performed to determine affinity and specificity of the molecules to the respective receptors. Table 1C lists the affinity of the respective molecules to FGFR3 and FGFR1 as measured by BIAcore® and/or FACS. In a binding assay to FGFR3-expressing cells, the IC₅₀ of the molecules was calculated (Example 6). Domain specificity was determined as described in Example 8. The ligand-independent inhibition of FGFR3 (neutralizing activity) was determined as described in Example 10. Finally, the molecules were synthesized in a number of different formats including Fab, miniantibody (Fab-dHLX), IgG1, IgG4, IgG3 and as single chain Fv (scFv).

TABLE 1C

Clone	Affinity to FGFR3 BIAcore nM	Affinity to FGFR3 (FACS) nM	Affinity to FGFR1 nM	K _{off} (s ⁻¹)	IC ₅₀ FR3 (FGF9 nM)	Domain Specificity	Ligand independent inhibition of FGFR3	Available formats
MSPRO59	1.5	<1	—	7.1 × 10e-4	19	2	+	Fab, Fab-dHLX IgG1, IgG4, mIgG3, scFv
MSPRO2	37	43	—	2 × 10e-2	360	2	~	Fab, Fab-dHLX, IgG1, IgG4,
MSPRO12	14	6.5	—	2.3 × 10e-3	58	2	+	Fab, Fab-dHLX, IgG1, IgG4, scFv
MSPRO11	4	4	108	4 × 10e-4	220	3		Fab, Fab-dHLX
MSPRO21	9	1.1	—	3.6 × 10e-3	50	3c		Fab, Fab-dHLX
MSPRO24	10		—	5.4 × 10e-3	70	3c		Fab, IgG1
MSPRO26	4	1.4	32	5 × 10e-4	70	3		Fab, Fab-dHLX
MSPRO28	9	0.3	160	4 × 10e-3	50	3		Fab
MSPRO29	6	<1	29	1.4 × 10e-3	20	3c	-	Fab, IgG1, IgG4, Fab-dHLX, scFv
MSPRO54	3.7	NA	2.5	2 × 10e-3	45	3c		Fab, IgG
MSPRO55	2.9	NA	—	7.4 × 10e-4	34	3c		Fab

Key: affinity (as measured in nM) of the respective molecules to FGFR3 and FGFR1 was measured by BIAcore® and/or FACS. IC₅₀ were determined for the dimeric dHLX format of certain molecules having an antigen binding site in an FDCP-FGFR3 proliferation assay performed with FGF9. Fab-dHLX refers to a Fab mini-antibody format where a dimer of the Fab monomer is produced as a fusion protein after insertion into an expression vector.

[0132] BIAcore® results for certain molecules

[0133] The numbers in Table 1D represent the IC₅₀s of the dimeric dHLX format of certain binders (molecule with antigen binding site) in the FDCP-FGFR3 proliferation assay performed with FGF9. The numbers in parentheses are the IC₅₀ of the monomeric Fabs in the same assay. Table 1E

MS-Pro-59-VH (SEQ ID NO: 113)	
1	QVQLQQSGPG LVKPSQTLSL TCAISGDSVS SNSAAWNWR QSPGRGLEWL

presents the K_D value for certain MSPRO molecules in miniantibody form, as determined in the BIAcore® assay.

TABLE 1D

binder	IC_{50}
MSPRO2	61 nM (360)
MSPRO12	26 nM (58)
MSPRO21	20 nM (50)
MSPRO26	8 nM (70)

[0134]

TABLE 1E

K_D determination for certain molecules		
Clone	BIAcore K_D [nM]	Number of measurements
MS-Pro-2-dHLX-MH	4.3 (37)	1
MS-Pro-11-dHLX-MH	0.7 (4)	1
MS-Pro-12-dHLX-MH	1.2 (14)	1
MS-Pro-21-dHLX-MH	2.2 (4.1)	1
MS-Pro-24-dHLX-MH	2 (10)	1
MS-Pro-26-dHLX-MH	2 (4)	1
MS-Pro-28-dHLX-MH	1.6 (9)	1

[0135] Certain non-limiting embodiments of molecules according to the present invention that block constitutive (ligand-independent) activation of FGFR3 are referred to herein MSPRO2, MSPRO12 and MSPRO59 comprising VH-CDR3 and VL-CDR3 domains having SEQ ID NO:8 and SEQ ID NO:9; SEQ ID NO:12 and SEQ ID NO:13; and SEQ ID NO:24 and SEQ ID NO:25, respectively. The preferred, but non-limiting, embodiments of molecules according to the present invention that block ligand-dependent activation of FGFR3 are referred to herein MSPRO11, MSPRO21, MSPRO24, MSPRO26, MSPRO29, and MSPRO54 comprising VH-CDR3 and VL-CDR3 domains having SEQ ID NO:10 and SEQ ID NO:11; SEQ ID NO:14 and SEQ ID NO:15; SEQ ID NO:16 and SEQ ID NO:17, SEQ ID NO:18 and SEQ ID NO:19; SEQ ID NO:21 and SEQ ID NO:22; SEQ ID NO:23 and SEQ ID NO:24, respectively. An antibody or a molecule of the present invention is said to have increased affinity for a RPTK if it binds a soluble dimeric form of said RPTK with a K_D of less than about 50 nM, preferably less than about 30 nM and more preferably less than about 10 nM, as determined by the BIAcore® chip assay for affinity, by a FACS-Scatchard analysis or other methods known in the art.

[0136] For convenience, Tables 1F and 1G outline the pairs of molecules according to their peptide SEQ ID NO and nucleotide SEQ ID NO, respectively.

TABLE 1F

Peptide pairs				
fragment				
antibody #	V heavy chain CDR3	V light chain CDR3	V heavy chain	V light chain
MSPRO2	SEQ ID NO: 8	SEQ ID NO: 9	SEQ ID NO: 103	SEQ ID NO: 92
MSPRO12	SEQ ID NO: 12	SEQ ID NO: 13	SEQ ID NO: 105	SEQ ID NO: 94
MSPRO59	SEQ ID NO: 24	SEQ ID NO: 25	SEQ ID NO: 113	SEQ ID NO: 102
MSPRO11	SEQ ID NO: 10	SEQ ID NO: 11	SEQ ID NO: 104	SEQ ID NO: 93
MSPRO21	SEQ ID NO: 14	SEQ ID NO: 15	SEQ ID NO: 106	SEQ ID NO: 95
MSPRO24	SEQ ID NO: 16	SEQ ID NO: 17	SEQ ID NO: 107	SEQ ID NO: 96
MSPRO26	SEQ ID NO: 18	SEQ ID NO: 19	SEQ ID NO: 108	SEQ ID NO: 97
MSPRO28	SEQ ID NO: 26	SEQ ID NO: 27	SEQ ID NO: 109	SEQ ID NO: 98
MSPRO29	SEQ ID NO: 20	SEQ ID NO: 21	SEQ ID NO: 110	SEQ ID NO: 99
MSPRO54	SEQ ID NO: 22	SEQ ID NO: 23	SEQ ID NO: 111	SEQ ID NO: 100
MSPRO55	SEQ ID NO: 28	SEQ ID NO: 29	SEQ ID NO: 112	SEQ ID NO: 101

[0137]

TABLE 1G

Nucleotide pairs				
fragment				
antibody #	V heavy chain CDR3	V light chain CDR3	V heavy chain	V light chain
MSPRO2	SEQ ID NO: 30	SEQ ID NO: 31	SEQ ID NO: 84	SEQ ID NO: 74
MSPRO12	SEQ ID NO: 34	SEQ ID NO: 35	SEQ ID NO: 89	SEQ ID NO: 75
MSPRO59	SEQ ID NO: 50	SEQ ID NO: 51	SEQ ID NO: 91	SEQ ID NO: 76
MSPRO11	SEQ ID NO: 32	SEQ ID NO: 33	SEQ ID NO: 85	SEQ ID NO: 70
MSPRO21	SEQ ID NO: 36	SEQ ID NO: 37	SEQ ID NO: 78	SEQ ID NO: 67
MSPRO24	SEQ ID NO: 38	SEQ ID NO: 39	SEQ ID NO: 79	SEQ ID NO: 64
MSPRO26	SEQ ID NO: 40	SEQ ID NO: 41	SEQ ID NO: 86	SEQ ID NO: 71
MSPRO28	SEQ ID NO: 42	SEQ ID NO: 43	SEQ ID NO: 80	SEQ ID NO: 62
MSPRO29	SEQ ID NO: 44	SEQ ID NO: 45	SEQ ID NO: 87	SEQ ID NO: 65
MSPRO54	SEQ ID NO: 46	SEQ ID NO: 47	SEQ ID NO: 82	SEQ ID NO: 73
MSPRO55	SEQ ID NO: 48	SEQ ID NO: 49	SEQ ID NO: 83	SEQ ID NO: 69

[0138] While the specific discovery of an antibody/molecule that blocks constitutive activation was made with respect to FGFR3 using a soluble dimeric form of FGFR3 to screen a phage display antibody library, it is believed that for all, or almost all, receptor protein tyrosine kinases, antibodies/molecules that block constitutive activation can be similarly obtained using a soluble dimeric form of a corresponding extracellular domain of a receptor protein tyrosine kinase. Non-limiting examples of receptor protein tyrosine kinases disclosed in Blume-Jensen et al. (2001) include EGFR/ErbB1, ErbB2/HER2/Neu, ErbB/HER3, ErbB4/HER4, IGF-1R, PDGFR- α , PDGFR- β , CSF-1R, kit/SCFR, Flk2/FH3, Flk1/VEGFR1, Flk1/VEGFR2, Flt4/VEGFR3, FGFR1, FGFR2/K-SAM, FGFR3, FGFR4, TrkA, TrkC, HGFR, RON, EphA2, EphB2, EphB4, Axl, TIE/TIE1, Tek/TIE2, Ret, ROSA1k, Ryk, DDR, LTK and MUSK.

[0139] Furthermore, antibodies/molecules that block ligand-dependent or ligand-independent activation of heterodimer receptor protein tyrosine kinases are intended to be included in the scope of the invention. Heterodimerization is well documented for members of the EGFR subfamily of receptor protein tyrosine kinases and others. Non-limiting examples include EGFR/PDGFR β , Flt1/KDR and EGFR/ErbB2 heterodimers. EGFR and PDGFR β heterodimers have been identified as a mechanism for PDGF signal transduction in rat vascular smooth muscle cells (Saito et al., 2001) and Flt-1/KDR heterodimers are required for vinculin assembly in focal adhesions in response to VEGF signaling (Sato et al., 2000).

[0140] The present invention is also directed to a molecule having the antigen-binding portion of an antibody which binds to a dimeric form of an extracellular portion of a receptor protein tyrosine kinase (RPTK), such as a FGFR, and blocks the ligand-independent (constitutive) activation and/or ligand-dependent activation of the RPTK.

[0141] Further provided is a method for screening a molecule comprising the antigen-binding portion of an antibody which blocks ligand-independent or ligand-dependent activation of a receptor protein tyrosine kinase, comprising:

[0142] providing a library of antigen binding fragments;

[0143] screening a library of antigen binding fragments for binding to a dimeric form of a receptor protein tyrosine kinase;

[0144] identifying an antigen binding fragment which binds to the dimeric form of the receptor protein tyrosine kinase as a candidate molecule for blocking constitutive activation of the receptor protein tyrosine kinase; and

[0145] determining whether the candidate molecule blocks constitutive and or ligand-dependent activation of the receptor protein tyrosine kinase in a cell.

[0146] Antibodies

[0147] Antibodies, or immunoglobulins, comprise two heavy chains linked together by disulfide bonds and two light chains, each light chain being linked to a respective heavy chain by disulfide bonds in a "Y" shaped configuration. Proteolytic digestion of an antibody yields Fv (fragment variable comprising Fab domains) and Fc (fragment crystalline) domains. The antigen binding domains, Fab', include regions where the polypeptide sequence varies. The term F(ab')₂ represents two Fab' arms linked together by disulfide bonds. The central axis of the antibody is termed the Fc fragment. Each heavy chain has at one end a variable domain (V_H) followed by a number of constant domains (C_H). Each light chain has a variable domain (V_L) at one end and a constant domain (C_L) at its other end, the light chain variable domain being aligned with the variable domain of the heavy chain and the light chain constant domain being aligned with the first constant domain of the heavy chain (CH1). The variable domains of each pair of light and heavy chains form the antigen binding site. The domains on the light and heavy chains have the same general structure and each domain comprises four framework regions, whose sequences are relatively conserved, joined by three hypervariable domains known as complementarity determining regions (CDR1-3). These domains contribute specificity and affinity of the antigen binding site. The isotype of the heavy chain (gamma, alpha, delta, epsilon or mu) determines immunoglobulin class (IgG, IgA, IgD, IgE or IgM, respectively). The light chain is either of two isotypes (kappa, ? or lambda, ?) found in all antibody classes.

[0148] It should be understood that when the terms “antibody” or “antibodies” are used, this is intended to include intact antibodies, such as polyclonal antibodies or monoclonal antibodies (mAbs), as well as proteolytic fragments thereof such as the Fab or F(ab')₂ fragments. Further included within the scope of the invention are chimeric antibodies; human and humanized antibodies; recombinant and engineered antibodies, and fragments thereof. Furthermore, the DNA encoding the variable region of the antibody can be inserted into the DNA encoding other antibodies to produce chimeric antibodies (see, for example, U.S. Pat. No. 4,816,567). Single chain antibodies fall within the scope of the present invention. Single chain antibodies can be single chain composite polypeptides having antigen binding capabilities and comprising amino acid sequences homologous or analogous to the variable regions of an immunoglobulin light and heavy chain (linked V_H-V_L or single chain Fv (scFv)). Both V_H and V_L may copy natural monoclonal antibody sequences or one or both of the chains may comprise a CDR-FR construct of the type described in U.S. Pat. No. 5,091,513, the entire contents of which are incorporated herein by reference. The separate polypeptides analogous to the variable regions of the light and heavy chains are held together by a polypeptide linker. Methods of production of such single chain antibodies, particularly where the DNA encoding the polypeptide structures of the V_H and V_L chains are known, may be accomplished in accordance with the methods described, for example, in U.S. Pat. No. 4,946,778, U.S. Pat. No. 5,091,513 and U.S. Pat. No. 5,096,815, the entire contents of which are hereby incorporated by reference.

[0149] Additionally, CDR grafting may be performed to alter certain properties of the antibody molecule including affinity or specificity. A non-limiting example of CDR grafting is disclosed in U.S. Pat. No. 5,225,539.

[0150] A “molecule having the antigen-binding portion of an antibody” as used herein is intended to include not only intact immunoglobulin molecules of any isotype and generated by any animal cell line or microorganism, but also the antigen-binding reactive fraction thereof, including, but not limited to, the Fab fragment, the Fab' fragment, the F(ab')₂ fragment, the variable portion of the heavy and/or light chains thereof, Fab miniantibodies (see WO 93/15210, U.S. patent application Ser. No. 08/256,790, WO 96/13583, U.S. patent application Ser. No. 08/817,788, WO 96/37621, U.S. patent application Ser. No. 08/999,554, the entire contents of which are incorporated herein by reference) and chimeric or single-chain antibodies incorporating such reactive fraction, as well as any other type of molecule or cell in which such antibody reactive fraction has been physically inserted, such as a chimeric T-cell receptor or a T-cell having such a receptor, or molecules developed to deliver therapeutic moieties by means of a portion of the molecule containing such a reactive fraction. Such molecules may be provided by any known technique, including, but not limited to, enzymatic cleavage, peptide synthesis or recombinant techniques.

[0151] The term “Fc” as used herein is meant as that portion of an immunoglobulin molecule (Fragment crystallizable) that mediates phagocytosis, triggers inflammation and targets Ig to particular tissues; the Fc portion is also important in complement activation.

[0152] In one embodiment of the invention, a chimera comprising a fusion of the extracellular domain of the RPTK and an immunoglobulin constant domain can be constructed useful for assaying for ligands for the receptor and for screening for antibodies and fragments thereof.

[0153] The “extracellular domain” when used herein refers the polypeptide sequence of the RPTKs disclosed herein which are normally positioned to the outside of the cell. The extracellular domain encompasses polypeptide sequences in which part or all of the adjacent (C-terminal) hydrophobic transmembrane and intracellular sequences of the mature RPTK have been deleted. Thus, the extracellular domain-containing polypeptide can comprise the extracellular domain and a part of the transmembrane domain. Alternatively, in the preferred embodiment, the polypeptide comprises only the extracellular domain of the RPTK. The truncated extracellular domain is generally soluble. The skilled practitioner can readily determine the extracellular and transmembrane domains of a RPTK by aligning the RPTK of interest with known RPTK amino acid sequences for which these domains have been delineated. Alternatively, the hydrophobic transmembrane domain can be readily delineated based on a hydrophobicity plot of the polypeptide sequence. The extracellular domain is N-terminal to the transmembrane domain.

[0154] The term “epitope” is meant to refer to that portion of any molecule capable of being bound by an antibody or a fragment thereof which can also be recognized by that antibody. Epitopes or antigenic determinants usually consist of chemically active surface groupings of molecules such as amino acids or sugar side chains and have specific three-dimensional structural characteristics as well as specific charge characteristics.

[0155] An “antigen” is a molecule or a portion of a molecule capable of being bound by an antibody which is additionally capable of inducing an animal to produce antibody capable of binding to an epitope of that antigen. An antigen may have one or more than one epitope. The specific reaction referred to above is meant to indicate that the antigen will react, in a highly selective manner, with its corresponding antibody and not with the multitude of other antibodies which may be evoked by other antigens.

[0156] A “neutralizing antibody” as used herein refers to a molecule having the antigen binding site to a specific receptor capable of reducing or inhibiting (blocking) activity or signaling through a receptor, as determined by in vivo or in vitro assays, as per the specification.

[0157] A monoclonal antibody (mAb) is a substantially homogeneous population of antibodies to a specific antigen. MAbs may be obtained by methods known to those skilled in the art. See, for example Kohler et al (1975); U.S. Pat. No. 4,376,110; Ausubel et al (1987-1999); Harlow et al (1988); and Colligan et al (1993), the contents of which references are incorporated entirely herein by reference. The mAbs of the present invention may be of any immunoglobulin class including IgG, IgM, IgE, IgA, and any subclass thereof. A hybridoma producing an mAb may be cultivated in vitro or in vivo. High titers of mAbs can be obtained in vivo production where cells from the individual hybridomas are injected intraperitoneally into pristine-primed Balb/c mice to produce ascites fluid containing high concentrations of the desired mAbs. MAbs of isotype IgM or IgG may be purified

from such ascites fluids, or from culture supernatants, using column chromatography methods well known to those of skill in the art.

[0158] Chimeric antibodies are molecules, the different portions of which are derived from different animal species, such as those having a variable region derived from a murine mAb and a human immunoglobulin constant region. Antibodies which have variable region framework residues substantially from human antibody (termed an acceptor antibody) and complementarity determining regions substantially from a mouse antibody (termed a donor antibody) are also referred to as humanized antibodies. Chimeric antibodies are primarily used to reduce immunogenicity in application and to increase yields in production, for example, where murine mAbs have higher yields from hybridomas but higher immunogenicity in humans, such that human/murine chimeric mAbs are used. Chimeric antibodies and methods for their production are known in the art (Better et al, 1988; Cabilly et al, 1984; Harlow et al, 1988; Liu et al, 1987; Morrison et al, 1984; Boulianne et al, 1984; Neuberger et al, 1985; Sahagan et al, 1986; Sun et al, 1987; Cabilly et al; European Patent Applications 125023, 171496, 173494, 184187, 173494, PCT patent applications WO 86/01533, WO 97/02671, WO 90/07861, WO 92/22653 and U.S. Pat. No. 5,693,762, U.S. Pat. No. 5,693,761, U.S. Pat. No. 5,585,089, U.S. Pat. No. 5,530,101 and U.S. Pat. No. 5,225,539). These references are hereby incorporated by reference.

[0159] Besides the conventional method of raising antibodies in vivo, antibodies can be generated in vitro using phage display technology. Such a production of recombinant antibodies is much faster compared to conventional antibody production and they can be generated against an enormous number of antigens. In contrast, in the conventional method, many antigens prove to be non-immunogenic or extremely toxic, and therefore cannot be used to generate antibodies in animals. Moreover, affinity maturation (i.e., increasing the affinity and specificity) of recombinant antibodies is very simple and relatively fast. Finally, large numbers of different antibodies against a specific antigen can be generated in one selection procedure. To generate recombinant monoclonal antibodies one can use various methods all based on phage display libraries to generate a large pool of antibodies with different antigen recognition sites. Such a library can be made in several ways: One can generate a synthetic repertoire by cloning synthetic CDR3 regions in a pool of heavy chain germline genes and thus generating a large antibody repertoire, from which recombinant antibody fragments with various specificities can be selected. One can use the lymphocyte pool of humans as starting material for the construction of an antibody library. It is possible to construct naive repertoires of human IgM antibodies and thus create a human library of large diversity. This method has been widely used successfully to select a large number of antibodies against different antigens. Protocols for bacteriophage library construction and selection of recombinant antibodies are provided in the well-known reference text, *Current Protocols in Immunology*, Colligan et al (Eds.), John Wiley & Sons, Inc. (1992-2000), Chapter 17, Section 17.1.

[0160] Another aspect of the present invention is directed to a method for screening for the antibody or molecule of the present invention by screening a library of antibody fragments displayed on the surface of bacteriophage, such as

disclosed in the Examples herein and described in WO 97/08320, U.S. Pat. No. 6,300,064 and Knappik et al. (2000), for binding to a soluble dimeric form of a receptor protein tyrosine kinase. An antibody fragment which binds to the soluble dimeric form of the RPTK used for screening is identified as a candidate molecule for blocking ligand-dependent activation and/or constitutive activation of the RPTK in a cell. Preferably the RPTK of which a soluble dimeric form is used in the screening method is a fibroblast growth factor receptor (FGFR), and most preferably FGFR3.

[0161] As a first screening method, the soluble dimeric form of a receptor tyrosine kinase can be constructed and prepared in a number of different ways. For instance, the extracellular domain of a RPTK joined to Fc and expressed as a fusion polypeptide that dimerizes naturally by means of the Fc portion of the RPTK-Fc fusion. Other suitable types of constructs of FGFR3, serving as guidance for other RPTKs, are disclosed in the Examples presented herein.

[0162] The assays for determining binding of antibody fragments to FGFR3, binding affinities, inhibition of cell proliferation, etc., are also described in the Examples herein below.

[0163] The term "cell proliferation" refers to the rate at which a group of cells divides. The number of cells growing in a vessel can be quantified by a person skilled in the art when that person visually counts the number of cells in a defined volume using a common light microscope. Alternatively, cell proliferation rates can be quantified by laboratory apparatus that optically or conductively measure the density of cells in an appropriate medium.

[0164] A second screen for antibody fragments as candidate molecules can be done using cells having very high overexpression of the RPTK, such as for instance RCJ-M15 cells overexpressing mutant (achondroplasia) FGFR3. In cells expressing very high levels of receptor some ligand-independent activation occurs even without the presence of a mutation, such as a constitutive activation mutation. It is believed that RPTK overexpression forces RPTKs to dimerize and signal even in the absence of ligand. These cells have monomeric receptors as well as dimeric receptors present on their cell surface. Using this type of cell, one of skill in the art would be able to identify all different kinds of antibodies, i.e., blocking ligand-dependent activation, blocking constitutive activation, blocking activation and binding only to monomeric form, etc.

[0165] Other screens can be carried out on cell lines expressing a RPTK carrying a mutation, such as the FDCP-FR3ach line expressing the FGFR3 achondroplasia mutation. The receptors of this line become constitutively active, e.g. are able to signal in the absence of a ligand as determined by ERK (MAPK) phosphorylation, a downstream effector.

[0166] A further aspect of the present invention relates to a method for treating or inhibiting a skeletal dysplasia or craniosynostosis disorder associated with constitutive activation of a RPTK which involves administering the molecule of the present invention to a subject need thereof. Non-limiting examples of skeletal dysplasias include achondroplasia, thanatophoric dysplasia (TDI or TDII), hypochondroplasia, and severe achondroplasia with developmental delay and acanthosis nigricans (SADDAN) dysplasia.

Non-limiting examples of craniosynostosis disorder are Muenke coronal craniosynostosis and Crouzon syndrome with acanthosis nigricans. The symptoms and etiology of these diseases and disorders are reviewed in Vajo et al. (Vajo et al, 2000).

[0167] The present invention also provides for a method for treating or inhibiting a cell proliferative disease or disorder associated with the action of an abnormal constitutively activated RPTK, for example tumor formation, primary tumors, tumor progression or tumor metastasis. A molecule comprising at least one antigen binding portion of an antibody that blocks constitutive activation of a RPTK is administered to a subject in need thereof to treat or inhibit such a cell proliferative disease or disorder.

[0168] The terms “treating or inhibiting a proliferative disease or disorder” or “treating or inhibiting a tumor” are used herein and in the claims to encompass tumor formation, primary tumors, tumor progression or tumor metastasis.

[0169] Tumor formation or tumor growth are intended to encompass solid and non-solid tumors. Solid tumors include mammary, ovarian, prostate, colon, cervical, gastric, esophageal, papillary thyroid, pancreatic, bladder, colorectal, melanoma, small-cell lung and non-small-cell lung cancers, granulose cell carcinoma, transitional cell carcinoma, vascular tumors, all types of sarcomas, e.g. osteosarcoma, chondrosarcoma, Kaposi's sarcoma, myosarcoma, heman-giosarcoma, and glioblastomas.

[0170] Non-solid tumors include for example hematopoi-etic malignancies such as all types of leukemia, e.g. chronic myelogenous leukemia (CML), acute myelogenous leukemia (AML), mast cell leukemia, chronic lymphocytic leukemia (CLL) and acute lymphocytic leukemia (ALL), lymphomas, and multiple myeloma (MM). FGFR3 has been implicated in poor prognosis seen in some patients.

[0171] Tumor progression is the phenomenon whereby cancers become more aggressive with time. Progression can occur in the course of continuous growth, or when a tumor recurs after treatment and includes progression of transitional cell carcinoma, osteo or chondrosarcoma, multiple myeloma, and mammary carcinoma (one of the known RPTKs involved in mammary carcinoma is ErbB3).

[0172] The role of the FGFR3 in tumor progression associated with transitional cell carcinoma and multiple myeloma has recently been elucidated (Cappellen, et al, 1999; Chesi, et al, 2001)

[0173] In another aspect of the present invention, molecules which bind FGFR, more preferably FGFR3, and block ligand-dependent receptor activation are provided. These molecules are useful in treating hyperproliferative diseases or disorders and non-neoplastic angiogenic pathologic conditions such as neovascular glaucoma, proliferative retinopathy including proliferative diabetic retinopathy, macular degeneration, hemangiomas, angiofibromas, and psoriasis. The role of FGFs and their receptors in neo- and hypervascularization has been well documented (Frank, 1997; Gerwins et al, 2000)

[0174] In another aspect of the present invention, the pharmaceutical compositions according to the present invention is similar to those used for passive immunization of humans with other antibodies. Typically, the molecules of

the present invention comprising the antigen binding portion of an antibody will be suspended in a sterile saline solution for therapeutic uses. The pharmaceutical compositions may alternatively be formulated to control release of active ingredient (molecule comprising the antigen binding portion of an antibody) or to prolong its presence in a patient's system. Numerous suitable drug delivery systems are known and include, e.g., implantable drug release systems, hydrogels, hydroxymethylcellulose, microcapsules, liposomes, microemulsions, microspheres, and the like. Controlled release preparations can be prepared through the use of polymers to complex or adsorb the molecule according to the present invention. For example, biocompatible polymers include matrices of poly(ethylene-co-vinyl acetate) and matrices of a polyanhydride copolymer of a stearic acid dimer and sebacic acid (Sherwood et al, 1992). The rate of release of the molecule according to the present invention, i.e., of an antibody or antibody fragment, from such a matrix depends upon the molecular weight of the molecule, the amount of the molecule within the matrix, and the size of dispersed particles (Saltzman et al., 1989 and Sherwood et al., 1992). Other solid dosage forms are described in (Ansel et al., 1990 and Gennaro, 1990).

[0175] The pharmaceutical composition of this invention may be administered by any suitable means, such as orally, intranasally, subcutaneously, intramuscularly, intravenously, intra-arterially, intralesionally or parenterally. Ordinarily, intravenous (i.v.) or parenteral administration will be preferred.

[0176] It will be apparent to those of ordinary skill in the art that the therapeutically effective amount of the molecule according to the present invention will depend, inter alia, upon the administration schedule, the unit dose of molecule administered, whether the molecule is administered in combination with other therapeutic agents, the immune status and health of the patient, the therapeutic activity of the molecule administered and the judgment of the treating physician. As used herein, a “therapeutically effective amount” refers to the amount of a molecule required to alleviate one or more symptoms associated with a disorder being treated over a period of time.

[0177] Although an appropriate dosage of a molecule of the invention varies depending on the administration route, age, body weight, sex, or conditions of the patient and should be ultimately determined by the physician, in the case of oral administration, the daily dosage can generally be between about 0.01-200 mg, preferably about 0.01-10 mg, more preferably about 0.1-10 mg, per kg body weight. In the case of parenteral administration, the daily dosage can generally be between about 0.001-100 mg, preferably about 0.001-1 mg, more preferably about 0.01-1 mg, per kg body weight. The daily dosage can be administered, for example, in regimens typical of 1-4 individual administrations daily. Various considerations in arriving at an effective amount are described, e.g., in Goodman and Gilman's: The Pharmacological Basis of Therapeutics, 8th ed., Pergamon Press, 1990; and Remington's Pharmaceutical Sciences, 17th ed., Mack Publishing Co., Easton, Pa., 1990.

[0178] The molecule of the present invention as an active ingredient is dissolved, dispersed or admixed in an excipient that is pharmaceutically acceptable and compatible with the active ingredient as is well known. Suitable excipients are,

for example, water, saline, phosphate buffered saline (PBS), dextrose, glycerol, ethanol, or the like and combinations thereof. Other suitable carriers are well-known to those in the art. (See, for example, Ansel et al., 1990 and Gennaro, 1990). In addition, if desired, the composition can contain minor amounts of auxiliary substances such as wetting or emulsifying agents, or pH buffering agents.

[0179] Combination Therapy

[0180] The combined treatment of one or more of the molecules of the invention with an anti-neoplastic or anti-chemotherapeutic drug such as doxorubicin, cisplatin or taxol provides a more efficient treatment for inhibiting the growth of tumor cells than the use of the molecule by itself. In one embodiment, the pharmaceutical composition comprises the antibody and carrier with an anti-chemotherapeutic drug.

[0181] The present invention also provides for an isolated acid molecule, which comprises a polynucleotide sequence encoding the molecule having at least one antigen binding portion of an antibody that blocks ligand-dependent activation and/or constitutive activation of a receptor protein tyrosine kinase such as FGFR3, and a host cell comprising this nucleic acid molecule. Furthermore, also within the scope of the present invention is a nucleic acid molecule containing a polynucleotide sequence having at least 90% sequence identity, preferably about 95%, and more preferably about 97% identity to the above encoding nucleotide sequence as would well understood by those of skill in the art.

[0182] The invention also provides isolated nucleic acid molecule that hybridizes under high stringency conditions to polynucleotides having SEQ ID NO:30 through SEQ ID NO:51 and SEQ ID NOS: 62, 64-65, 67, 69-71, 73-76 78-80, 82-87, 89, 91 or the complement thereof. As used herein, highly stringent conditions are those which are tolerant of up to about 5-20% sequence divergence, preferably about 5-10%. Without limitation, examples of highly stringent (-10° C. below the calculated T_m of the hybrid) conditions use a wash solution of 0.1 $\times$ SSC (standard saline citrate) and 0.5% SDS at the appropriate T_i below the calculated T_m of the hybrid. The ultimate stringency of the conditions is primarily due to the washing conditions, particularly if the hybridization conditions used are those which allow less stable hybrids to form along with stable hybrids. The wash conditions at higher stringency then remove the less stable hybrids. A common hybridization condition that can be used with the highly stringent to moderately stringent wash conditions described above is hybridization in a solution of 6 $\times$ SSC (or 6 $\times$ SSPE), 5 $\times$ Denhardt's reagent, 0.5% SDS, 100 μ g/ml denatured, fragmented salmon sperm DNA at an appropriate incubation temperature T_i . See generally Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 2d edition, Cold Spring Harbor Press (1989)) for suitable high stringency conditions.

[0183] Stringency conditions are a function of the temperature used in the hybridization experiment and washes, the molarity of the monovalent cations in the hybridization solution and in the wash solution(s) and the percentage of formamide in the hybridization solution. In general, sensitivity by hybridization with a probe is affected by the amount and specific activity of the probe, the amount of the target nucleic acid, the detectability of the label, the rate of

hybridization, and the duration of the hybridization. The hybridization rate is maximized at a T_i (incubation temperature) of 20-25 $^{\circ}$ C. below T_m for DNA:DNA hybrids and 10-15 $^{\circ}$ C. below T_m for DNA:RNA hybrids. It is also maximized by an ionic strength of about 1.5M Na^+ . The rate is directly proportional to duplex length and inversely proportional to the degree of mismatching.

[0184] Specificity in hybridization, however, is a function of the difference in stability between the desired hybrid and "background" hybrids. Hybrid stability is a function of duplex length, base composition, ionic strength, mismatching, and destabilizing agents (if any).

[0185] The T_m of a perfect hybrid may be estimated for DNA:DNA hybrids using the equation of Meinkoth et al (1984), as

$$T_m = 81.5^{\circ} \text{ C.} + 16.6(\log M) + 0.41(\% \text{ GC}) - 0.61(\% \text{ form}) - 500/L$$

and for DNA:RNA hybrids, as

$$T_m = 79.8^{\circ} \text{ C.} + 18.5(\log M) + 0.58(\% \text{ GC}) - 11.8(\% \text{ GC})^2 - 0.56(\% \text{ form}) - 820/L$$

[0186] where

[0187] M , molarity of monovalent cations, 0.01-0.4 M NaCl,

[0188] $\% \text{ GC}$, percentage of G and C nucleotides in DNA, 30%-75%,

[0189] $\% \text{ form}$, percentage formamide in hybridization solution, and

[0190] L , length hybrid in base pairs.

[0191] T_m is reduced by 0.5-1.5 $^{\circ}$ C. (an average of 1 $^{\circ}$ C. can be used for ease of calculation) for each 1% mismatching.

[0192] The T_m may also be determined experimentally. As increasing length of the hybrid (L) in the above equations increases the T_m and enhances stability, the full-length rat gene sequence can be used as the probe.

[0193] Filter hybridization is typically carried out at 68 $^{\circ}$ C., and at high ionic strength (e.g., 5-6 $\times$ SSC), which is non-stringent, and followed by one or more washes of increasing stringency, the last one being of the ultimately desired high stringency. The equations for T_m can be used to estimate the appropriate T_i for the final wash, or the T_m of the perfect duplex can be determined experimentally and T_i then adjusted accordingly.

[0194] The present invention also relates to a vector comprising the nucleic acid molecule of the present invention. The vector of the present invention may be, for example, a plasmid, cosmid, virus, bacteriophage or another vector used conventionally in genetic engineering, and may comprise further genes such as marker genes which allow for the selection of said vector in a suitable host cell and under suitable conditions.

[0195] Furthermore, the vector of the present invention may, in addition to the nucleic acid sequences of the invention, comprise expression control elements, allowing proper expression of the coding regions in suitable hosts. Such control elements are known to the artisan and may

include a promoter, a splice cassette, translation initiation codon, translation and insertion site for introducing an insert into the vector.

[0196] Preferably, the nucleic acid molecule of the invention is operatively linked to said expression control sequences allowing expression in eukaryotic or prokaryotic cells.

[0197] Control elements ensuring expression in eukaryotic or prokaryotic cells are well known to those skilled in the art. As mentioned herein above, they usually comprise regulatory sequences ensuring initiation of transcription and optionally poly-A signals ensuring termination of transcription and stabilization of the transcript.

[0198] Methods for construction of nucleic acid molecules according to the present invention, for construction of vectors comprising said nucleic acid molecules, for introduction of said vectors into appropriately chosen host cells, for causing or achieving the expression are well-known in the art (see, e.g., Sambrook et al., 1989; Ausubel et al., 1994).

[0199] The invention also provides for conservative amino acid variants of the molecules of the invention. Variants according to the invention also may be made that conserve the overall molecular structure of the encoded proteins. Given the properties of the individual amino acids comprising the disclosed protein products, some rational substitutions will be recognized by the skilled worker. Amino acid substitutions, i.e. "conservative substitutions," may be made, for instance, on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues involved.

[0200] For example: (a) nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan, and methionine; (b) polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine; (c) positively charged (basic) amino acids include arginine, lysine, and histidine; and (d) negatively charged (acidic) amino acids include aspartic acid and glutamic acid. Substitutions typically may be made within groups (a)-(d). In addition, glycine and proline may be substituted for one another based on their ability to disrupt α -helices. Similarly, certain amino acids, such as alanine, cysteine, leucine, methionine, glutamic acid, glutamine, histidine and lysine are more commonly found in α -helices, while valine, isoleucine, phenylalanine, tyrosine, tryptophan and threonine are more commonly found in β -pleated sheets. Glycine, serine, aspartic acid, asparagine, and proline are commonly found in turns. Some preferred substitutions may be made among the following groups: (i) S and T; (ii) P and G; and (iii) A, V, L and I. Given the known genetic code, and recombinant and synthetic DNA techniques, the skilled scientist readily can construct DNAs encoding the conservative amino acid variants.

[0201] As used herein, "sequence identity" between two polypeptide sequences indicates the percentage of amino acids that are identical between the sequences. "Sequence similarity" indicates the percentage of amino acids that either are identical or that represent conservative amino acid substitutions.

[0202] Conjugates

[0203] One embodiment of the present invention provides molecules of the present invention conjugated to cytotoxins. The cytotoxic moiety of the antibody may be a cytotoxic drug or an enzymatically active toxin or bacterial or plant origin, or an enzymatically active fragment of such a toxin including, but not limited to, diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from *Pseudomonas aeruginosa*), ricin A chain, abrin A chain, modeccin A chain, α -sarcin, Aleurites fordii proteins, dianthin proteins, curcin, croton, saponin, gelonin, mitogellin, restrictocin, phenomycin, and enomycin. In another embodiment, the molecules of the present invention are conjugated to small molecule anti-cancer drugs. Conjugates of the antibody and such cytotoxic moieties are made using a variety of bifunctional protein coupling agents. Examples of such reagents include SPDP, IT, bifunctional derivatives of imidoesters such as dimethyl adipimidate HCl, active esters such as disuccinimidyl suberate, aldehydes such as glutaraldehyde, bis-azido compounds such as bis-(p-azido-benzoyl) hexanediamine, bis-diazonium derivatives, disocyanates and bis-active fluorine compounds. The lysing portion of a toxin may be joined to the Fab fragment of the antibodies.

[0204] Additionally, the molecules of the present invention can also be detected in vivo by imaging, for example imaging of cells which have undergone tumor progression or have metastasized. Antibody labels or markers for in vivo imaging of RPTKs include those detectable by X-radiography, NMR, PET, or ESR. For X-radiography, suitable labels include radioisotopes such as barium or cesium, which emit detectable radiation but are not overtly harmful to the subject. Suitable markers for NMR and ESR include those with a detectable characteristic spin, such as deuterium, which may be incorporated into the antibody.

[0205] A specific antibody or antibody portion which has been labeled with an appropriate detectable imaging moiety, such as a radioisotope (for example, ^{131}I , ^{111}In , ^{99}Tc), a radio-opaque substance, or a material detectable by nuclear magnetic resonance, is introduced (for example, parenterally, subcutaneously or intraperitoneally) into the mammal to be examined for a disorder. It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moieties needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries. The labeled antibody or antibody portion will then preferentially accumulate at the location of cells which contain a specific RPTK. In vivo tumor imaging is described in Burchiel et al., (1982).

[0206] The methods and compositions described herein may be performed, for example, by utilizing pre-packaged diagnostic test kits comprising in one or more containers (i) at least one immunoglobulin of the invention and (ii) a reagent suitable for detecting the presence of said immunoglobulin when bound to its target. A kit may be conveniently used, e.g., in clinical settings or in home settings, to diagnose patients exhibiting a disease (e.g., skeletal dysplasia, craniosynostosis disorders, cell proliferative diseases or disorders, or tumor progression), and to screen and identify those individuals exhibiting a predisposition to such disorder.

ders. A composition of the invention also may be used in conjunction with a reagent suitable for detecting the presence of said immunoglobulin when bound to its target, as well as instructions for use, to carry out one or more methods of the invention.

[0207] Having now generally described the invention, the same will be more readily understood through reference to the following examples, which are provided by way of illustration and are not intended to be limiting of the present invention.

EXAMPLES

[0208] An important approach to controlling cellular FGFR3 activity is the generation of reagents that block receptor signaling. Without wishing to be bound by theory, molecules which bind the extracellular domain of the receptor may inhibit the receptor by competing with FGF or heparin binding or, alternatively, by preventing receptor dimerization. Additionally, binding to the extracellular domain may accelerate receptor internalization and turnover. Humanized antibodies are expected to have inhibitory/neutralizing action and are of particular interest since they are considered to be valuable for therapeutic applications, especially by avoiding the human anti-mouse antibody response frequently observed with rodent antibodies. The experiments in which the neutralizing antibodies are screened, identified and obtained using fully synthetic human antibody libraries (for discovering highly specific binders against a wide variety of antigens) and FGFR3 extracellular domain are described below.

Example 1

[0209] Attempt to Generate Anti-FGFR3 Antibodies

[0210] One hundred micrograms of soluble FGFR3 in complete Freund's Adjuvant were injected into Balb/c 3T3 naive mice (9 animals). Two repeated injections of 20 micrograms were performed at week intervals. 10 days after the second booster injection, blood was drawn from animals and serum was tested for the presence of polyclonal antibodies both by monitoring for binding to the receptor as well as for neutralizing activity at a dilution of 1:50. No significant neutralizing activity was observed in the tested serum (20% at most in some animals). A perfusion injection of 20 micrograms of soluble receptor was administered 1-2 days later but all the mice harboring some activity of neutralizing Ab died. The experiment was repeated twice with the same results.

Example 2

[0211] Generation of the FGFR3 Antigens

[0212] Two dimeric forms of the extracellular domain of the human FGFR3 were prepared for use as antigen. One was a histidine-tagged domain with a Serine 371 to Cysteine (S371C) substitution (thanatophoric dysplasia (TD) mutation) to facilitate dimerization and the second one an Fc fusion. The S371C variant was shown to bind heparin and FGF9 coated plates and to inhibit FGF9-dependent FDCP-FR3 proliferation. The Fc fusion was similarly effective in binding assays, demonstrating its potential as an inhibitor of FGFR function and as a target for selecting FGFR3 inhibitory molecules. Both soluble receptors were employed to select neutralizing human recombinant antibodies.

[0213] The two variants of the FGFR3 extracellular domain were prepared as follows:

[0214] 1. A construct containing the extracellular portion of FGFR3 with a thanatophoric dysplasia (TD) mutation to facilitate dimer formation conjugated to a His-tag (histidine tag) was generated. A bluescript plasmid comprising the human FGFR3 gene (pBS-hFGFR3) was used as template for PCR with the following primers:

5'-ACGTGCTAGC TGAGTCCTTG GGGACGGAGC (SEQ ID NO: 2)
AG.

5'-ACGTCTCGAG TTAATGGTGA TGGTGATGGT (SEQ ID NO: 3)
GTGCATACAC ACAGCCCCGCC TCCTC.

[0215] wherein the Ser 371 Cys (S371C) substitution is bold and underlined.

[0216] The nucleotide sequence encoding the extracellular domain of FGFR3 with the TD substitution is denoted herein SEQ ID NO:7.

[0217] The PCR fragment was digested with XhoI and ligated into pBlueScript digested with EcoRV and XhoI. The resulting plasmid, pBsFR3²³⁻³⁷⁴TDhis, was cleaved with NheI and XhoI and the DNA fragment encoding the extracellular domain of FGFR3 was ligated into the same restriction sites in pCEP-Pu/Ac7 (Yamaguchi et al., 1999; Kohfeldt et al., 1997), generating the pCEP-hFR3²³⁻³⁷⁴TDhis plasmid construct.

[0218] To express this FGFR3 variant, 293E cells (EBNA virus transfected 293 cells) were transfected with the aforementioned plasmid, pCEP-hFR3²³⁻³⁷⁴TDhis, clones were identified and grown. Cell supernatants analyzed by Western blot with anti-His antibody demonstrated high expression of the soluble receptor. Supernatants from large scale preparations were then subjected to batch affinity purification with Ni-NTA beads and the tagged soluble receptor was eluted by a step gradient ranging from 20 mM to 500 mM imidazol. A sample from each eluate was loaded onto a 7.5% SDS-PAGE and stained with GelCode (Pierce). In parallel, Western blot analysis was performed and analyzed with anti-His antibodies. SDS-PAGE (FIG. 1) and immunoblot (not shown) analyses demonstrated peak amounts of purified extracellular FGFR3 in the 2nd (2) 50 mM imidazol fraction. About 0.5 mg of pure protein was obtained following this single step purification. In FIG. 1, T=total protein, D= dialysed protein, U=unbound fraction.

[0219] To assess whether hFR3²³⁻³⁷⁴TDhis (hFR3-TDhis) retained the ability to associate with heparin and heparin-FGF complex, heparin coated wells were incubated with 2, 4 or 10 μ g purified (labeled as FR3 2, FR3 4 or FR3 10, respectively in FIG. 2) or unpurified (FR3 s) hFR3²³⁻³⁷⁴TDhis with (checkered bar) or without FGF9 (200 ng/well, hatched bar). The binding of hFR3²³⁻³⁷⁴TDhis to each well was determined with anti-His antibody. Mock supernatant (M sup), PBS and unpurified mouse FR3AP (FGFR3-alkaline phosphatase, labeled as mFRAP sup) were included as controls. The results, as presented in FIG. 2, demonstrated that, similar to what was reported for the wild-type receptor, hFR3²³⁻³⁷⁴TDhis binds to heparin and that this interaction is augmented by the presence of FGF9. Finally, it was demonstrated that hFR3²³⁻³⁷⁴TDhis inhibits FDCP-FR3 FGF-dependent proliferation in a dose dependent

dent manner. hFR3²³⁻³⁷⁴TDhis had no inhibitory effect on proliferation when FDCP-FR3 cells were grown in the presence of IL-3. Taken together, hFR3²³⁻³⁷⁴TDhis proved to be a good candidate as a target antigen for screening for FGFR3 neutralizing antibodies.

[0220] 2. The extracellular domain of FGFR3 and FGFR1 were prepared as Fc fusions (FR3exFc and FR1exFc). The amino acid sequence of FGFR3 (NCBI access no: NP_000133) is denoted herein SEQ ID NO:1.

[0221] To construct the FR3exFc fusion, a polynucleotide sequence (denoted herein SEQ ID NO:4) encoding the extracellular domain of FGFR3 was PCR amplified to contain terminal KpnI and BamHI restriction sites for insertion into the KpnI and BamHI sites of pCXFc (denoted herein SEQ ID NO:5). This insertion positions the extracellular domain of FGFR3 to be expressed as a fusion with the Fc amino acid sequence (denoted herein SEQ ID NO:6).

[0222] Both FR3exFc and FR1exFc soluble receptors were demonstrated to be expressed to a high level in transiently transfected 293T cells (T-cell antigen infected human embryonic kidney 293 cells). The observation that both soluble receptors remain bound to heparin-coated wells even following extensive washes led the laboratory of the present inventors to try to purify the proteins with the commercial heparin-Sepharose® resin (Pharmacia). One hundred ml volume supernatants, harvested 48 hours post-transfection with either FR3exFc or FR1exFc coding plasmids, were incubated overnight at 4° C. with 1 ml heparin-Sepharose® resin. The resin was washed and then subjected to PBS supplemented with increasing concentration of NaCl. Aliquots of each fraction were analyzed by 7.5% SDS-PAGE stained with GelCode® (Pierce) demonstrating a purification profile of more than 90% homogeneity and a peak elution at 400 mM NaCl for FR3exFc (FIG. 3; T=total protein, U=unbound fraction, W=wash). In contrast, FR1exFc was hardly retained on the resin. This result was confirmed by Western analysis of the same fractions with anti-FGFR1ex antibodies demonstrating that most of FR1exFc is in the unbound fraction (not shown).

[0223] Functional analysis of FR3exFc and FR1exFc showed that both compete efficiently for FGF9 binding and stimulating FGFR3, thus, demonstrating their potential as inhibitors of FGFRs function and as a target (FR3exFc) for selecting FGFR3 inhibitory molecules.

[0224] Neutralizing effect of soluble receptors

[0225] The ability of hFR3-TDhis and FR3exFc to inhibit FGF-dependent FDCP-R3 cell proliferation was compared. Both soluble receptors inhibited FDCP-R3 cell proliferation, however, FR3exFc was about 60 times more potent than hFR3TDhis. Neither had an effect on FDCP cells stimulated with IL-3. (FIG. 4; legend: ?-FR3²³⁻³⁷⁴TDhis on FDCP-FR3 cells+FGF9, |-FR3exFc on FDCP-FR3 cells+FGF9, ?-FR3²³⁻³⁷⁴TDhis on FDCP-FR3 cells+IL-3, X-FR3exFc on FDCP-FR3 cells+IL-3). The fact that FR3exFc is entirely in dimeric form whereas only a small proportion (1/10) of hFR3²³⁻³⁷⁴TDhis is in dimeric form might explain, at least in part, this difference.

Example 3

- [0226] Screening for Antibodies
- [0227] Panning and first screening of Ab Binding Characterization
- [0228] The screening strategies to identify Fabs from the Human Combinatorial Antibody Library (HuCAL®, developed at MorphoSys, Munich, Germany and disclosed in WO 97/08320, U.S. Pat. No. 6,300,064, and Knappik et al., (2000), the entire contents of which are incorporated herein by reference, using soluble dimeric forms of the extracellular domain of the FGFR3 receptor are shown in Table 2.

TABLE 2

Panning Strategies			
	Panning Round 1	Panning Round 2	Panning Round 3
Screen 1	FR3-TDhis	HEK293	FR3-TDhis
Screen 2	FR3exFc	RCJ-FR3ach	FR3exFc
	captured with mouse anti-human IgG		captured with mouse anti-human IgG
Screen 3	FR3-TDhis (Round 1 of panning 1)	RCJ-FR3ach & RCJ-FR3wt	FR3exFc
			Captured with mouse anti-human IgG

[0229] The screening was carried out, for example in Screen 1, by coating the wells of a 96 well plate with hFR3²³⁻³⁷⁴TDhis (FR3-TDhis), panning with the bacteriophage library and selecting the positive clones. The positive clones were then tested on HEK293 (293, human embryonic kidney) cells, expressing endogenous FGFR3. The positive clones were selected and rescreened on FR3-TDhis. Two additional similar screenings were carried out as shown in Table 2. In screen 2 the first and third pannings were carried out with the FR3exFc antigen and the second panning carried out with RCJ cells expressing a mutant (achondroplasia) form of FGFR3. An overview of the number of initial hits and of the candidate clones is shown in Table 3.

TABLE 3

Overview of Screenings 1, 2 and 3 on FGFR3				
	screened clones	primary hits	sequenced clones	consolidated candidate clones (ELISA & FACS)
Screen 1	1076	208	69	15 (MSPRO 1-15)
Screen 2	864	300	32	22 (MSPRO 20-33 and 52-59)
Screen 3	768	487	52	11 (MSPRO 40-50)

[0230] Sequence and Vector Data

[0231] A plasmid map of the dHLX-MH vector having SEQ ID NO:52 is presented in FIG. 28. FIG. 29 shows the plasmid map of the phage display vector, having SEQ ID NO:53, used in accordance with the present invention.

[0232] FIG. 30 displays the polynucleotide sequences of the specific V_L and V_H domains of MSPRO2 (SEQ ID NO:74 and 84); MSPRO11 (SEQ ID NO:70 and 85);

MSPRO12 (SEQ ID NO:75 and 89); MSPRO21 (SEQ ID NO:67 and 78); MSPRO24 (SEQ ID NO:64 AND 79); MSPRO26 (SEQ ID NO:71 AND 86); MSPRO28 (SEQ ID NO:62 AND 80); MSPRO29 (SEQ ID NO:65 AND 87); MSPRO54 (SEQ ID NO:73 AND 82); MSPRO55 (SEQ ID NO:69 AND 83); and MSPRO59 (SEQ ID NO:76 AND 91). The sequences include the framework domains 1-4 and the CDR domains 1-3. SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:66, SEQ ID NO:68, and SEQ ID NO:73 denote herein the polynucleotide sequences of the parent V_L (kappa or lambda) strands. SEQ ID NO:77, SEQ ID NO:81, SEQ ID NO:88 and SEQ ID NO:90 denote herein the polynucleotide sequences of the V_H parent strands.

Example 4

[0233] Analysis of Fabs Identified in First Screening

[0234] Specificity of Antibody recognition

[0235] The first screening yielded 15 different Fabs that specifically recognize FGFR3 in vitro and on the cell surface. Fourteen of these were further analysed. LY6.3, an anti-lysosome antibody, was isolated from the same library and serves as a control. ELISA analysis, according to the following protocol was carried out to determine the specificity of the isolated Fabs for FGFR3 or FGFR1.

[0236] Fab-FR3/Fc Binding Assay

[0237] MaxiSorp® ELISA plates were coated with 100 μ l anti-human Fc (10 μ g/ml) in bicarbonate overnight at 4° C. Wells were washed five consecutive times with a PBS solution containing 0.1% Tween 20 (PBST). The well surface was blocked with 250 μ l PBST+3% BSA (blocking solution) for 1 hour at 37° C. This was followed by capturing 1 μ g of FGFR/Fc for 1 hour at room temperature. To assess the antibody binding to the captured FGFR/Fc, 1 μ g each of the tested Fabs was incubated in 100 μ l blocking solution per well 1 hour at room temperature. Wells were washed 5 times with PBST. Reaction was initiated with the addition of 100 μ l of 0.8 μ g/ml goat anti-human Fab-HRP (horseradish peroxidase) diluted in blocking solution, subsequently washed and detected with TMB substrate (Pierce). The absorbance was measured at 450 nm. A comparison of ELISA analyses done in both laboratories, Prochon and MorphoSys, is presented in FIG. 27 and in Table 4.

TABLE 4

	ProChon		MorphoSys	
	FR1/Fc	FR3/Fc	FR1/Fc	FR3/Fc
MS-PRO1	++	++	+/-	+
MS-PRO2	-	++	-	++
MS-PRO3	+	++	-	++
MS-PRO4	-	+	-	++
MS-PRO5	-	++	+/-	+
MS-PRO6	-	++	-	+
MS-PRO7	-	++	-	+
MS-PRO8	+	++	-	+
MS-PRO9	-	+/-	+/-	+
MS-PRO10	+	++	-	++
MS-PRO11	-	+/-	+	++
MS-PRO12	-	+/-	-	++
MS-PRO13	-	+/-	+/-	+
MS-PRO14	-	-	-	+
LY6.3 (control)	-	-		

[0238] In most cases, the data generated in both laboratories are in agreement. However, some Fabs behave differently. For example, MS-PRO3 and MS-PRO-10 were found to be completely FGFR3-specific under certain conditions while under other conditions both show considerable cross-reaction with FGFR1. Subsequent FACS analysis supported the cross reactivity for MS-PRO3, but not for MS-PRO10. Taking into account the potency and specificity of the Fabs, MS-PRO2 had the highest score according to these preliminary data.

Example 5

[0239] Affinity of Fab to FGFR3

[0240] The affinity measurements were performed by BIAcore® analysis according to the standard procedure recommended by the supplier (Pharmacia). The anti-Fc antibody was coupled via the EDC/NHS chemistry to the chip and subsequently FGFR3 was captured. The Fabs of the invention were then bound to this surface.

[0241] Table 5 shows a comparison of affinities of Fabs candidates to FGFR3 as determined by BIAcore® and by FACS-scatchard.

TABLE 5

Comparison of Antibody Affinities to FGFR3 determined by BIAcore® and FACS-Scatchard		
Fab clone	BIAcore® [nM]	Indirect FACS-Scatchard [nM]
MSPRO2	37 $\pm$ 10	43
MSPRO11	4 $\pm$ 2	4
MSPRO12	14 $\pm$ 2	6.5
MSPRO21	9 $\pm$ 2	0.6
MSPRO24	10 $\pm$ 2	0.3
MSPRO26	4 $\pm$ 1	1.4
MSPRO28	9 $\pm$ 0.4	0.3
MSPRO29	6 $\pm$ 4	0.4

[0242] Table 1E (in the Detailed Description, vide supra) shows the affinity as determined by BIAcore® for the Fab candidates shown in Table 5 converted into the Fab mini-antibody format, Fab-dHLX-MH, where a dimer of the Fab monomer is produced after insertion into an expression vector as a fusion protein.

[0243] Table 6 shows the results of a competition assay wherein each MSPRO Fab was bound to FGFR3 at a concentration of 500 nM or 1,000 nM and coinjected in pairs with the other MSPRO Fabs. The (-) indicates binding to the same or nearby epitope while (+) indicates binding to different epitope. The results show that MSPRO2 and 12 bind to the same or nearby epitope while MSPRO 11, 21, 24, 26, 28 and 29 bind to an epitope different from that of MSPRO 2 or 12.

TABLE 6

	2	11	12	21	24	26	28	29
2		+	-	+	+	+	+	+
11	+		+	-	-	-	-	-
12	-	+		+	+	+	+	+
21	+	-	+		-	-	-	-
24	+	-	+	-		-	-	-

TABLE 6-continued

	2	11	12	21	24	26	28	29
26	+	-	+	-	-	-	-	-
28	+	-	+	-	-	-	-	-
29	+	-	+	-	-	-	-	-

Example 6

[0244] Specific Neutralizing Activity of the Antibodies**[0245]** A: FDCP Cell Proliferation Assay

[0246] The FDCP cell line is a murine immortalized, interleukin 3 (IL-3) dependent cell line of myelocytic bone marrow origin, which does not express endogenous FGF Receptors (FGFR). Upon transfection with FGFR cDNA, the FDCP cell line exhibits an FGF dose-dependent proliferative response that can replace the dependence on IL-3. FDCP cell lines, transfected with FGFR cDNAs can therefore be used to screen for specific inhibitors or activators of FGFR, as well as for analyzing FGFR signaling. The FDCP cell response to various ligands was quantitated by a cell proliferation assay with XTT reagent (Cell Proliferation Kit, Biological Industries Co.). The method is based on the capability of mitochondrial enzymes to reduce tetrazolium salts into soluble colored formazan compounds which can be quantitated and is indicative of cell viability. Specifically, FDCP cells expressing FGFR3IIIb, FGFR3IIIc or FGFR1 were grown in "full medium" (Iscove's Medium containing 2 ml glutamine, 10% FCS, 100 ug/ml penicillin, 100 ug/ml streptomycin) supplemented with 5 ug/ml heparin and 10 ng/ml FGF9. Cells were split every 3 days and kept in culture no more than one month. One day prior to the experiment, the cells were split. Before the experiment, the cells were washed 3 times (1000 rpm, 6 min) with full medium. Later, the cells were resuspended and counted with Trypan Blue. Twenty thousand (20,000) cells per well were added to wells in a 96-well plate in 50 ul in full medium containing 5 ug/ml heparin. Conditioned medium was added in an additional volume of 50 ul full medium containing FGF9 at varying concentrations to a final volume of 100 ul. A primary stock solution (usually 2x the higher concentration) of the antibody (or Fabs) was prepared in Iscove's+++ containing 5 μ g/ml heparin and 2.5 ng/ml FGF9 or IL-3 (final concentration 1.25 ng/ml). Dilutions were filtered in a 0.2 μ m syringe nitrocellulose filter blocked first with 1 mg/ml BSA and washed then with Iscove's+++ . Aliquots of requested serial dilutions were prepared. Dilutions were kept on ice until use. 50 μ l of the corresponding 2x final concentration was added to each well and the plate was incubated at 37° C. for either 40 hours or 64 hours. After incubation, the reaction was developed as follows: 100 μ l of activator solution was added to 5 ml XTT reagent and mixed gently. 50 μ l of mixture was added to each well. Optical density (OD) at 490 nm at this point gave the zero time reading.

[0247] Cells were then incubated at 37° C. for 4 hours (in the case of a 40-hour incubation) or 2 hours (in the case of a 64-hour incubation) and proliferation was measured by O.D. at 490 nm (A490).

[0248] It is noted that the assay is successful when the O.D. of untreated control growing with saturated amounts of

FGF (10 and 20 ng/ml) is at least 1.3 O.D. units. Furthermore, it is noted that the background of wells with no cells should be 0.2-0.35 O.D. units and that the O.D. absorbance of 1.25 ng/ml FGF9 should not be less than 40% of the O.D. absorbance achieved with saturated FGF 9 concentration (10 and 20 ng/ml). Specific inhibition of FGF and FGF receptor mediated proliferation should always be accompanied with lack of any inhibition of the same antibody concentration on IL-3 dependent cell proliferation.

[0249] The following FDCP cell lines were used:

[0250] *FDCP-C10 (C10): FDCP cells transfected with the human wild-type FGFR3IIIc.

[0251] *FDCP-R3: FDCP cells transfected with the human wild-type FGFR3IIIb.

[0252] *FDCP-R1: FDCP cells transfected with the human wild-type FGFR1. *FDCP-F3Ach: FDCP cells infected with human FGFR3 mutated at amino acid Glycine 380 to Arginine (G380R), analogous to the most common human achondroplasia mutation.

[0253] B: Neutralizing activity

[0254] The neutralizing activity of the antibodies was measured by the aforementioned cell proliferation analysis in FDCP-FR3 and FDCP-FR1 cell lines and is presented in **FIG. 5**. Increasing amounts of the indicated Fabs (MSPRO 2, 3 and 4) were added to FDCP-FR3 (closed triangle ? (2), star * (3), and circle ? (4)) or FDCP-FR1 (open triangle ? (2), open square ? (3) and open circle ? (4)) grown in the presence of FGF9. Two days later, an XTT proliferation assay was performed. While none of the Fabs inhibited FDCP-FR1 cell proliferation, MSPRO2 (?) and MSPRO3 (*) inhibited FDCP-FR3 proliferation with a similar IC50 of about 1.0 μ g/ml. In contrast, MSPRO4 (?) had no inhibitory effect on FDCP-FR3 proliferation. The rest of the Fabs, MSPRO 1, 3, 5, 6, 7, 9, 11, 12, 13, 14, were similarly analyzed on FDCP-FR3 expressing cells. Increasing amounts of the indicated Fabs were added to FDCP-FR3 grown in the presence of FGF9 (**FIG. 6**). Inhibitors of FGFR3 signaling were antibodies MSPRO 1, 3, 5, 7, 9, 11, 12. The results of the proliferation assay done at two sites are compared in Table 7 (NA=data not available).

TABLE 7

	Prochon		MorphoSys	
	FDCP-FR1	FDCP-FR3	FDCP-FR1	FDCP-FR3
MSPRO1	-	++	NA	NA
MSPRO2	-	++	NA	++
MSPRO3	-	++	NA	++
MSPRO4	-	-	NA	-
MSPRO5	-	+	NA	+
MSPRO6	-	-	NA	+/-
MSPRO7	-	++	NA	+
MSPRO8	-	+/-	NA	+/-
MSPRO9	-	+	NA	+
MSPRO10	-	+	NA	NA
MSPRO11	-	+++	NA	++
MSPRO12	-	+++	NA	+++
MSPRO13	-	-	NA	NA
MSPRO14	-	-	NA	NA
LY6.3	-	-	NA	NA

[0255] As shown in Table 7, there is an excellent agreement between the data. About half of the Fabs show con-

siderable neutralizing activity, MSPRO12 being the most potent. Most of the inhibitory Fabs performed well in the binding assay (Table 4), with MSPRO11 and MSPRO12 being the exception to the rule, however, clearly remain good candidates to pursue. None of the Fabs (including those that crossreact with FGFR1) inhibited FGF-dependent FDCP-FR1 proliferation. In addition, FDCP-FR3 cells grown in the presence of IL-3 were not affected by any of the Fabs.

[0256] An additional 20 new Fabs were selected from the second panning. Three of these new Fabs were subjected to the FDCP cell proliferation test and all were found to neutralize the receptor (MSPRO52 (?), MSPRO54 (?) and MSPRO55 (*) in **FIG. 7A**). Interestingly and in accord with MorphoSys affinity data, one Fab (MSPRO54) showed strong neutralizing activity against FGFR1 (**FIG. 7B**). MSPRO29 (?) and a control antibody Ly6.3 (i) were also tested in this assay.

Example 7

[0257] Receptor Expression and Activation in RCJ Cells

[0258] RCJ cell assay

[0259] RCJ cells (fetal rat calvaria-derived mesenchymal cells, RCJ 3.1C5.18; Grigoriadis, 1988) were generated to express various FGF Receptors in an inducible manner, in the absence of tetracycline. The M14 line (RCJ-FR3ach) expresses FGFR3-ach380 mutant upon induction by the removal of tetracycline. The cells were incubated in low serum after which FGF was added to stimulate receptor function and signaling. The cells were lysed and the receptor level, receptor activation and signaling are assessed by Western with anti-active ERK (or JNK) (Promega). The lysates is immunoprecipitated with anti-FGFR3 (Santa Cruz), and a Western immunoblots is performed using anti-phospho-tyrosine (Promega) antibodies. W11 refers to the RCJ cells expressing wild type FGFR3. RCJ-FR1 and RCJ-FR2 refer to RCJ cells expressing the FGFR1 and FGFR2 receptors, respectively. **FIG. 21** provides a flow chart of the experimental procedure.

[0260] The transfected RCJ cells were grown in a-MEM supplemented with 15% fetal calf serum, 1xpenicillin/streptomycin/nystatin, 1xglutamine, 600 µg/ml neomycin, 2 µg/ml tetracycline, 50 µg/ml hygromycin B to subconfluence. The medium was aspirated off and the cells washed with trypsin, 1 ml/10 cm dish, then trypsinized with 0.5 ml/10 cm dish. The cells were resuspended in 10 ml a-MEM supplemented with 15% fetal calf serum, 1xpenicillin/streptomycin/nystatin, 1xglutamine, 600 µg/ml neomycin, and 2 µg/ml tetracycline.

[0261] Six hundred thousand (6×10^6) cells/well were seeded in a 6-well dish. The cells were washed thrice 24 hours later (or 8 hours later if twice the amount of cells are seeded) with 1 ml a-MEM, and then incubated with a-MEM supplemented with 15% fetal calf serum, 1xpenicillin/streptomycin/nystatin, and 1xglutamine (induction medium) for 16 hours. Cells were washed thrice with 1 ml a-MEM and allowed to grow for 4 additional hours in 1 ml of 0.5% exhausted serum (prepared by diluting the induction medium X30 with a-MEM).

[0262] FGF9 (1 ng/ml) was added for 5 minutes and the cells placed on ice. The cells were washed twice with

ice-cold PBS and lysed with 0.5 ml lysis buffer. The cells were scraped into an Eppendorf tube, vortexed once and placed on ice for 10 minutes. The lysate was microcentrifuged for 10 minutes at 4° C., and the cleared lysate was transferred into a fresh Eppendorf tube.

[0263] The protein content was determined by Bradford or DC protein assay (Bio-Rad, cat# 500-0116) following manufacture instructions. Total protein aliquots, supplemented with 1/5 volume of 5xsample buffer, were boiled for 5 minutes and stored at -20° C. until ready to load on gel. In parallel an immunoprecipitation (IP) assay was performed, 10 µl anti-FGFR3 antibodies were added to the rest of the lysates and incubated for 4 hours at 4° C. Twenty (20) µl protein A-Sepharose® was added and incubated for 1 hour at 4° C. with continuous shaking. Afterwards, the mixture was microcentrifuged 15 seconds, and the fluid was aspirated, carefully leaving a volume of ~30 µl above the beads. The beads were washed 3 times with 1 ml lysis buffer. At this step, the protease inhibitor mix was omitted from the buffer.

[0264] After the final wash, 15 µl of 5xsample buffer was added, samples were boiled 5 minutes and stored at -20° C. until ready to load onto gel. Samples were loaded onto a 7.5% SDS-PAGE, cast on a Mini-PROTEAN II electrophoresis cell, and run at 100 V through the upper gel and at 150 V through the lower gel. Proteins were transferred onto a nitrocellulose sheet using the Mini trans-blot electrophoretic transfer cell at 100 V for 75 minutes or at 15 V overnight. The lower part of the total lysate Western blots was probed with anti-active JNK (anti-phosphorylated Jun Kinase) and the upper part was probed with anti-FGFR3, both at 5×10^3 dilutions.

[0265] **FIG. 8A** shows that MSPRO2 blocks FGFR3 activation in W11 cells and weakly blocks signaling in M14 cells, and MSPRO12 blocks FGFR3 receptor activation in W11 and M14 expressing cells. Furthermore MSPRO13 appeared to be able to block FGFR1 activation while none of the Fabs blocked FGFR2 activation. **FIG. 8B** shows the inhibitory capacity of MSPRO12 and MSPRO59 on wild type FGFR3 expressing cells, as seen as reduction in JNK signaling. MSPRO29 strongly inhibits FGFR3 activation (<5 ug), MSPRO12 has an inhibitory effect but at a higher concentration (5-20 ug).

[0266] The IP lysate Western blots were probed with anti-phosphotyrosine (R&D Systems). Hybridization was detected by ECL following the manufacturer's instructions.

[0267] BIAcore® and proliferation analyses showed that among the new Fabs, MSPRO54 is highly cross reactive with FGFR1. To further test the cross reactivity of the new Fabs, RCJ cells expressing either FGFR3ach (RCJ-M14; M14 on **FIG. 9A**) FGFR3 wild type (W 11 on **FIG. 9B**), FGFR1 (R1-1 on **FIG. 9C**) or FGFR2 (R2-2 on **FIG. 9D**) were incubated with increasing amount of a control antibody LY6.3, MSPRO29, 54 and 59 for one hour. FGF9 was added for 5 minutes and cell lysates were analyzed by Western blot for ERK activation (phosphorylated ERK; pERK) (**FIGS. 9A-9D**). Furthermore, MSPRO13 was able to block FGFR1 activation while none of the Fabs blocked FGFR2 activation. **FIGS. 9A-9D** show the results of several Fabs, at different mg concentrations, on RCJ expressing wildtype FGFR3 or the different FGFR types. MSPRO29 appeared as the best FGFR3 blocker and was also effective in blocking FGFR1 (**FIG. 9c**); however, MSPRO54 was the most effective

tive Fab against FGFR1. None of the Fabs significantly inhibited FGFR2 activity. There are only a few amino acid residues within the third Ig domain that are shared by FGFR3 and FGFR1 but not by FGFR2. Making mutants at these sites should clarify their role in Fab-receptor binding.

Example 8

[0268] Epitope Mapping of Selected Fabs

[0269] Constructs comprising cDNAs that code for segments of the extracellular domain of FGFR3 were generated (**FIG. 10**). D2 comprises Ig domain 2, D2-3 comprises Ig domains 2 and 3, and D1-3 comprises Ig domains 1, 2 and 3. These constructs include pChFR3^{D2}Fc that codes for Ig-like domain 2 of FGFR3 and pChFR3^{D2,3}Fc that codes for domain 2 and 3, both as human Fc fusions. The corresponding chimeric proteins, as well as the control hFR3exFc (containing domains 1, 2 and 3) were anchored to an ELISA plate coated with a human Fc antibody. A panel of 8 best Fabs, MSPRO2, 11, 12, 21, 24, 26, 28 and 29, were added, and bound Fab was determined with HRP-a human Fab (**FIG. 11**). The results in **FIG. 11** demonstrate that MSPRO2 (speckled bar) and MSPRO12 (diagonally hatched bar) differ from the other tested Fabs. Both bind to the Ig like domain 2 while the others require domain 3 for binding. It was then tested whether or not Fabs that belong to the second group would distinguish the FGFR3IIIc isoform from the FGFR3IIIb from. FDCP-FR3IIIb or FDCP-FR3IIIc cells were incubated in the presence of 1.25 ng/ml FGF9 with increasing doses of either MSPRO12 or MSPRO29. Ly6.3 was included as control. After 2 days in culture, cell proliferation was measured with the XTT reagent. Clearly, MSPRO29 (open triangle) was completely ineffective against the IIIb isoform (**FIG. 12**). In contrast, MSPRO12 (square on hatched or solid lines) was equally effective against both isoforms. These data suggest that residues that differ between the two isoform are critical for MSPRO29 (and probably also for the other Fabs in the same group) FGFR3 binding.

[0270] Domains in FGFR3 recognized by the new Fabs

[0271] From the data presented, MSPRO antibodies can be divided into 2 groups, one that includes Fabs that bind the FGFR3 Ig II domain (MSPRO2 and 12) and a second with members that require the Ig III domain for binding (MSPRO11, 21, 24, 26, 28, and 29). To classify the new Fabs obtained from the last screen, as well as some previously obtained Fabs, a proliferation assay of FDCP cells expressing either FR3IIIb or FR3IIIc was performed. The cells were incubated in the presence of 10 (IIIb) or 5 (IIIc) ng/ml FGF9 with increasing doses of the indicated Fabs. After 2 days in culture, cell proliferation was measured with the XTT reagent.

[0272] The data shows that MSPRO59 (*) efficiently inhibited both FDCP-FR3IIIb (**FIG. 13A**) and FDCP-FR3IIIc cells (**FIG. 13B**), while MSPRO21, 24, 26, 28, 29 and 54 inhibited FDCP-FR3IIIc proliferation only.

Example 9

[0273] Bone Culture

[0274] Radiolabeled MSPRO29 was used to determine whether MSPRO Fabs are able to penetrate the bone growth plate.

[0275] To determine the effect of iodination on Fab activity, 50 μ g of MSPRO29 was first labeled with cold iodine

using Pierce IodoGen coated tubes. The process was carried out either without iodine, with 0.04 mM NaI (low I) or with 1 mM NaI (high I). MSPRO29 was then purified through a Sephadex® G-50 column. The ability of the modified Fab to bind FGFR3 was determined by ELISA. MaxiSorp® wells were coated with anti-human Fc. FGFR3/Fc was then anchored to the wells. In parallel, a similar set of wells was left in blocking buffer only (no FR3/Fc, hatched bars). The unmodified (no I) or the modified MSPRO29 (low or high, 2 G-50 fractions each, 1 and 2) were added at approximately 5 μ g/well and binding was measured with anti-human Fab. Fresh MSPRO29 and buffer alone were included as controls (**FIG. 14**: FGFR3/Fc, checkered bars; no FGFR3/Fc, hatched bars). MS-PRO29 was labeled with 1 mCi ¹²⁵I. The specific activity of the Fab was 17 μ Ci/ μ g.

[0276] MSPRO29 labeled in the presence of 0.04 mM NaI showed equal binding to the receptor as compared to the control unmodified Fab. MSPRO29 labeled in the presence of 1 mM NaI (high I, 1 and 2) also bound the receptor, however, the noise level of this sample was as high as the signal itself suggesting that at the high iodide concentration the Fab was inactivated.

[0277] The neutralizing activity of the modified Fab was tested in a proliferation assay using FDCP-FR3 (C10) (**FIG. 15**). FDCP-FR3 (C10) cells were treated with the indicated amount of labeled or unlabeled (without I) MSPRO29. The proliferation rate of the cells was determined by XTT analysis. The Fab was labeled at either 0.04 mM (Low) or 1 mM NaI (High). Two G-50 fractions (1 and 2) were analyzed. Fresh MSPRO29 and buffer alone (mock) were included as controls.

[0278] This experiment showed that MSPRO29, labeled at 0.04 mM NaI, maintained its inhibiting activity almost entirely while MSPRO29 labeled at 1 mM NaI had indeed lost its activity completely.

[0279] Ex vivo distribution of ¹²⁵I MSPRO29 in bone culture

[0280] Femora prepared from newborn mice were incubated with 2 μ g ¹²⁵I-MSPRO29 (17 μ Ci/ μ g) or ¹²⁵I-Ly6.3 (20 μ Ci/ μ g) for 1, 3 or 5 days in culture. Then, sections were processed for radiomicroscopy. After 3 days in culture, MSPRO29 was predominantly visualized at the higher hypertrophic zone and to a lesser extent at the secondary ossification region (**FIGS. 16A-16F**). Hematoxylin-eosin staining of growth plate treated with radiolabelled MSPRO29 or Ly6.3 (**FIGS. 16A and 16D**, respectively) $\times$ 100 magnification. Radiomicroscopic sections of growth plate treated with radiolabelled MS-PRO29 or Ly6.3 (**FIGS. 16B and 16E**) at $\times$ 100 magnification. **FIGS. 16C and 16F** are the same as **FIGS. 16B and 16E**, respectively, but at $\times$ 400 magnification. The arrows in **FIGS. 16B and 16C** indicate the location of the specific binding of the radiolabelled MSPRO29 to the upper hypertrophic zone of the growth plate.

[0281] As compared to MSPRO29, the control Ly6.3 Fab was weakly and evenly distributed throughout the whole growth plate. At day 1 in culture, the signal was weaker but with similar distribution pattern. This distribution also holds at 5 days in culture with a less favorable signal to noise ratio

(data not shown). This clearly demonstrates that MSPRO29 binds FGFR3 in our target organ.

Example 10

[0282] Neutralization of Constitutively Active Receptors

[0283] The inhibitory activity of MSPRO antibodies on ligand-dependent and ligand-independent FDCP proliferation expressing FGFR3 Achondroplasia mutation was tested.

[0284] A proliferation assay was carried out using FDCP-FR3wt (C10, **FIG. 17A**) or FDCP-FR3ach cells (**FIG. 17B**) incubated with 1.25 or 5 ng/ml FGF9 respectively and with increasing amounts of MSPRO54 or MSPRO59. As shown in **FIG. 17**, both MSPRO54 (diamond ?) and 59 (square !) antibodies neutralize the mutant receptor. FDCP-FR3ach acquired ligand independent cell proliferation due to the high expression of the FGFR3ach mutation. MSPRO29 (?) inhibits the FDCP-FR3wt activity at a level similar to MSPRO54 and 59 but is less effective in inhibiting the FGFR3ach receptor in this assay system.

[0285] FDCP cells that express the achondroplasia FGFR3 (FDCP-FR3ach) and proliferate independently of ligand were incubated with the indicated amount of MSPRO12, 29, 59 or the control LY6.3. Two days later, cell proliferation was determined by an XTT analysis. When inhibition of cell proliferation by the MS-PRO 12, 29, 54 and 59 were tested, only the antibodies 12 and 59 (the only Ab which recognized D2 domain) inhibited the ligand-independent cell proliferation (**FIGS. 18A and 18B**).

Example 11

[0286] RCS Chondrocyte Culture

[0287] Effect of Fabs on growth arrest of RCS Chondrocytes

[0288] RCS is a rat chondrosarcoma derived cell line expressing preferentially high levels of FGFR2 and FGFR3 and low levels of FGFR1 (Sahni, 1999). In this cell line FGFR functions as an inhibitor of cell proliferation similar to its expected role in the achondroplasia phenotype. Analysis of RCS cell proliferation mediated by the addition of different molecules of the invention, showed that MSPRO54 and MSPRO59 were able to restore cell proliferation.

[0289] The screening was performed on RCS parental cells in 96 well plates. Cells were seeded at a concentration of 2,000 cells/well. The following day 10 ng/ml FGF-9 and 5 µg/ml heparin were added to the cells. 50 µg/ml of the antibodies were added. Positive and negative controls for cell proliferation are included in this assay at the same concentrations as the tested molecules. On the fourth day of incubation, plates were observed under the microscope. If all cells were viable, no quantitative assay to measure the effect of the variants was performed. If cell death was observed, the Cy-Quant assay kit is used to measure the amount of the cells. The results are measured in a fluoro ELISA reader. **FIG. 19** shows the ELISA results in bar graph form. Untreated cells are shown speckled, ligand treated cells are shown in gray, control antibody (LY6.3) treated cells are in black while MSPRO54 and MSPRO59 treated cells are shown in diagonally hatched or checkered bars, respectively.

Example 12

[0290] Ex Vivo Bone Culture

[0291] The femoral bone cultures were performed by excising the hind limbs of 369-mice, heterozygous or

homozygous mice for the achondroplasia G369C mutation (age P0). The limbs were carefully cleaned up from the surrounding tissue (skin and muscles) and the femora exposed. The femora were removed and further cleared from tissue remains and ligaments. The femora were measured for their initial length, using a binocular with an eyepiece micrometer ruler. The bones were grown in 1 ml of medium in a 24 well tissue culture dish. The growing medium is a-MEM supplemented with penicillin (100 units/ml), streptomycin (0.1 mg/ml) and nystatin (12.5 units/ml). In addition, the medium contains BSA (0.2%), α-glycerophosphate (1 mM) and freshly prepared ascorbic acid (50 µg/ml). The bones were cultured for 15 days. Measurements of bone length and medium replacement were performed every three days.

[0292] At the end of the experiment, the growth rate of the bones was determined. The growth rate of bones is calculated from the slope of a linear regression fit on the length measurements obtained from day 3 to 9.

[0293] The result, as shown in **FIG. 20**, demonstrate a dose dependent increase in the growth rate of bones treated with MS-PRO 59 in comparison to non-relevant control LY6.3 Fab. The LY6.3-treated control femurs, marked with a circle, grew at the slowest rate. The MSPRO59 treated femurs exhibited a higher growth rate, with the optimal rate achieved at the highest MSPRO59 concentration of 400 µg/ml (square), which can be seen as the steeper slope. Moreover, the growth rates achieved by 400 microgram/ml of MSPRO59 doubled in comparison to the control Ab (3.55 U/day as compared to 1.88 U/day, respectively). This experiment shows the neutralizing effect of the MSPRO59 antibody on an ach mutant FGFR3, in an ex vivo model.

Example 13

[0294] In-vivo Trials

[0295] FDCP-FR3ach cells, but not FDCP (control) cells, were found to be tumorigenic when injected into nude mice. Each of 9 mice received two sub-cutaneous injections with different amount of transfected cells. Fourteen days after injection, progressively growing tumors started to appear at the site of FDCP-FR3ach injection but not at the FDCP site of injection. External examination of the tumors showed a high vascular capsule. ¹²⁵I-labeled MSPRO59 and LY6.3 were injected I.P. into nude mice carrying the FDCP-FR3ach derived tumor. The tumors were dissected 4 and 24 hours later and radioactivity was measured. Concentration of labeled MSPRO59 Abs in FDCP-FR3ach derived tumors is shown in **FIG. 22**.

Example 14

[0296] Animal Model for Bladder Carcinoma

[0297] Recent studies have shown that the IIIb isoform of FGFR3 is the only form expressed in bladder carcinoma, in particular an FGFR3 with an amino acid substitution wherein Serine 249 is replaced by Cysteine (S249C). The progression of the cancer is believed to be a result of the constitutive activation resulting from this amino acid substitution. In order to create the FGFR3 IIIb form, we isolated the IIIb region of FGFR3 from HeLa cells and generated a

full length FGFR3IIIb isoform in pLXSN. Retroviruses, expressing either normal FGFR3 (FR3wt) or mutant FGFR3 (FR3-S249C) were produced and used to infect FDCP cells. Stable pools were generated and further used for in-vitro and in-vivo experiments.

[0298] MSPRO59 reduces tumor size in mice

[0299] Twelve nude mice were injected with 2×10^6 FDCP-S249C cells subcutaneous at 2 locations, one on each flank. A week later MSPRO59 was administered I.P. at 400 μ g per mouse (3 mice in total), followed by 3 injections of 275 μ g each, in 2 to 3 days intervals. Following 24 and 26 days the tumor size was measures. **FIG. 23** shows the inhibitory effect of MSPRO59 on tumor size.

[0300] Treatment of FDCP-S249C-derived tumors with MSPRO59

[0301] Nude mice (3 in each group), were injected subcutaneous at 2 locations, one on each flank, with 2×10^6 FDCP-S249C cells each. A week later, 400 or 80 μ g MSPRO59 were injected IP. Three days later, mice were injected with 400 μ g followed by 5 additional injections with 275 μ g MSPRO59, each, every 3 or 4 days. Mice initially treated with 80 μ g MSPRO59 were similarly given an additional 80 μ g MSPRO59 followed by 5 injections with 50 μ g MSPRO59 at the same schedule. Mice injected with PBS were used as control. Tumor volume was estimated from measurements in 3 dimensions at 16, 20, 23 or 32 days post cell injection. As shown in **FIG. 24** there is both a delay in tumor appearance and an inhibitory effect on tumor progression in the treated mice. This indicates that these FGFR3 inhibitors are potent in vivo.

[0302] These data may also help us understand the mechanism by which the S249C-derived tumors were developed. Since we are using pools of cells, treatment with MSPRO59 inhibited the susceptible cells, leading to delay in tumor appearance. However, over time, the resistant cells survived and proliferated, giving rise to a solid tumor.

[0303] MSPRO59 inhibits FDCP-FR3ach380 derived tumor growth.

[0304] Nude mice were injected subcutaneously in the flank with 2×10^6 FDCP-FR3ach380 cells, each. Treatment with MSPRO59 began at the day of tumor appearance. Three mice were treated with a known tyrosine kinase inhibitor (TKI -50 mg/Kg/injection) and three with 400 μ g followed by 3 additional injections with 300 μ g MSPRO59, every 3 or 4 days. Three mice were treated with PBS alone as control. The tumor size was estimated as before at the indicated days after cell injection. The dose schedule is shown in Table 8 below.

TABLE 8

	Days After FDCP-FR3ach380 Cell Injection			
	21	25	28	31
MSPRO59 (μ g)	400 μ g	300 μ g	300 μ g	300 μ g
PBS (μ l)	50	50	50	50

[0305] Results are shown in bar graph format in **FIG. 25A**.

[0306] MSPRO59 inhibits FDCP-S249C induced tumor growth

[0307] After several months in culture FDCP-S249C cells acquire partial resistance to MSPRO antibodies and eventually become completely insensitive. To overcome the instability of the FDCP-derived pools, clones from a pool of FDCP-S249C cells were isolated and characterized. These clones were tested in an XTT proliferation assay and were shown to be inhibited by MSPRO59. 2×10^6 cells from each clone were injected into nude mice. Tumors appeared 18-30 after injection.

[0308] FDCP-S249C clone cells were injected subcutaneously on the flank. A week later mice were injected with 280 μ g MSPRO59 single chain (SC) I.P. every day. Mice injected with PBS were used as control. Tumor volume was estimated from measurements in 3 dimensions at 18 or 24 days post cell injection. An apparent inhibition of tumor growth by MSPRO59 (ScFv) was observed in tumors derived from clone 2 (**FIG. 26**). **FIG. 25B** shows the inhibition effected by MSPRO59scFv and MSPRO59 Fab compared to the control. Both inhibit growth of the tumor resulting from constitutively activated cells.

[0309] Having now fully described this invention, it will be appreciated by those skilled in the art that the same can be performed within a wide range of equivalent parameters, concentrations, and conditions without departing from the spirit and scope of the invention and without undue experimentation.

[0310] While this invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications. This application is intended to cover any variations, uses, or adaptations of the inventions following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth as follows in the scope of the appended claims.

[0311] All references cited herein, including journal articles or abstracts, published or corresponding U.S. or foreign patent applications, issued U.S. or foreign patents, or any other references, are entirely incorporated by reference herein, including all data, tables, figures, and text presented in the cited references. Additionally, the entire contents of the references cited within the references cited herein are also entirely incorporated by references. Reference to known method steps, conventional methods steps, known methods or conventional methods is not in any way an admission that any aspect, description or embodiment of the present invention is disclosed, taught or suggested in the relevant art.

[0312] The foregoing description of the specific embodiments will so fully reveal the general nature of the invention that others can, by applying knowledge within the skill of the art (including the contents of the references cited herein), readily modify and/or adapt for various applications such specific embodiments, without undue experimentation, without departing from the general concept of the present invention. Therefore, such adaptations and modifications are intended to be within the meaning and range of equivalents of the disclosed embodiments, based on the teaching and

guidance presented herein. It is to be understood that the phraseology or terminology herein is for the purpose of description and not of limitation, such that the terminology or phraseology of the present specification is to be interpreted by the skilled artisan in light of the teachings and guidance presented herein, in combination with the knowledge of one of ordinary skill in the art.

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<210> SEQ ID NO 6

<211> LENGTH: 235

<212> TYPE: PRT

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Fc domain of Immunoglobulin

<400> SEQUENCE: 6

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Asp Pro Glu Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro
1           5           10           15

```

```

Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro
20           25           30

```

```

Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr
35           40           45

```

```

Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn
50           55           60

```

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Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys	Pro	Arg
65				70					75					80	
Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	Thr	Val
			85					90					95		
Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser
		100					105					110			
Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys
		115				120					125				
Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	Ser	Arg	Asp
	130				135					140					
Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu	Val	Lys	Gly	Phe
145				150					155					160	
Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu
			165					170					175		
Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe
		180					185						190		
Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly
	195					200					205				
Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala	Leu	His	Asn	His	Tyr
	210				215						220				
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225				230					235						

<210> SEQ ID NO 7
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 <212> TYPE: DNA
 <213> ORGANISM: Homo sapiens
 <220> FEATURE:
 <221> NAME/KEY: mutation
 <222> LOCATION: (1046)..(1048)
 <223> OTHER INFORMATION: BASE PAIRS ENCODING THANATOPHORIC DYSPLASIA
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gccctcggag cgtgtcctgg tggggcccca gcggctgcag gtgctgaatg cctccacga	240
ggactccggg gcctacagct gccggcagcg gctcacgcag cgcgtactgt gccacttcag	300
tgtgcgggtg acagacgctc catcctcggg agatgacgaa gacggggagg acgaggctga	360
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gctgctggcc gtgccggccg ccaacaccgt ccgcttcgcg tgcccagccg ctggcaaccc	480
cactccctcc atctcctggc tgaagaacgg caggaggttc cgcggcgagc accgcattgg	540
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ccggcgcaac tacacctgcg tcgtggagaa caagtttggc agcatccggc agacgtacac	660
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ccagacggcg gtgctgggca gcgacgtgga gttccactgc aaggtgtaca gtgacgcaca	780
gccccacatc cagtggctca agcacgtgga ggtgaacggc agcaaggtgg gcccgacgg	840
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ggtttctctcc ttgcacaacg tcacctttga ggacgccggg gaggacacct gcctggcggg    960
caattctatt gggttttctc atcactctgc gtggctgggtg gtgctgccag ccgaggagga    1020
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<210> SEQ ID NO 8
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: CDR domain from phage library
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<400> SEQUENCE: 8
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```
Asp Phe Leu Gly Tyr Glu Phe Asp Tyr
1                5
```

```
<210> SEQ ID NO 9
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: CDR domain from phage library
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```
<400> SEQUENCE: 9
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```
Gln Ser Tyr Asp Tyr Ser Ala Asp Tyr
1                5
```

```
<210> SEQ ID NO 10
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: CDR domain from phage library
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```
<400> SEQUENCE: 10
```

```
Tyr Tyr Gly Ser Ser Leu Tyr His Tyr Val Phe Gly Gly Phe Ile Asp
1                5                10                15
```

```
Tyr
```

```
<210> SEQ ID NO 11
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: CDR domain from phage library
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<400> SEQUENCE: 11
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```
Gln Ser His His Phe Tyr Glu
1                5
```

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<210> SEQ ID NO 12
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: CDR domain from phage library
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<400> SEQUENCE: 12
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```
Tyr His Ser Trp Tyr Glu Met Gly Tyr Tyr Gly Ser Thr Val Gly Tyr
1                5                10                15
```

```
Met Phe Asp Tyr
                20
```

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<210> SEQ ID NO 13
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: CDR domain from phage library

<400> SEQUENCE: 13

Gln Ser Tyr Asp Phe Asp Phe Ala
1 5

<210> SEQ ID NO 14
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: CDR domain from phage library

<400> SEQUENCE: 14

Asp Asn Trp Phe Lys Pro Phe Ser Asp Val
1 5 10

<210> SEQ ID NO 15
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: CDR domain from phage library

<400> SEQUENCE: 15

Gln Gln Tyr Asp Ser Ile Pro Tyr
1 5

<210> SEQ ID NO 16
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: CDR domain from phage library

<400> SEQUENCE: 16

Val Asn His Trp Thr Tyr Thr Phe Asp Tyr
1 5 10

<210> SEQ ID NO 17
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: CDR domain from phage library

<400> SEQUENCE: 17

Gln Gln Met Ser Asn Tyr Pro Asp
1 5

<210> SEQ ID NO 18
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: CDR domain from phage library

<400> SEQUENCE: 18

Gly Tyr Trp Tyr Ala Tyr Phe Thr Tyr Ile Asn Tyr Gly Tyr Phe Asp

-continued

1 5 10 15

Asn

<210> SEQ ID NO 19
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: CDR domain from phage library

<400> SEQUENCE: 19

Gln Ser Tyr Asp Asn Asn Ser Asp Val
1 5

<210> SEQ ID NO 20
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: CDR domain from phage library

<400> SEQUENCE: 20

Thr Trp Gln Tyr Ser Tyr Phe Tyr Tyr Leu Asp Gly Gly Tyr Tyr Phe
1 5 10 15

Asp Ile

<210> SEQ ID NO 21
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: CDR domain from phage library

<400> SEQUENCE: 21

Gln Gln Thr Asn Asn Ala Pro Val
1 5

<210> SEQ ID NO 22
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: CDR domain from phage library

<400> SEQUENCE: 22

Asn Met Ala Tyr Thr Asn Tyr Gln Tyr Val Asn Met Pro His Phe Asp
1 5 10 15

Tyr

<210> SEQ ID NO 23
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: CDR domain from phage library

<400> SEQUENCE: 23

Gln Ser Tyr Asp Tyr Phe Lys Leu
1 5

<210> SEQ ID NO 24
<211> LENGTH: 8

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<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: CDR domain from phage library

<400> SEQUENCE: 24

Ser Tyr Tyr Pro Asp Phe Asp Tyr
1 5

<210> SEQ ID NO 25
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: CDR domain from phage library

<400> SEQUENCE: 25

Gln Ser Tyr Asp Gly Pro Asp Leu Trp
1 5

<210> SEQ ID NO 26
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: CDR domain from phage library

<400> SEQUENCE: 26

Gly Gly Gly Trp Val Ser His Gly Tyr Tyr Tyr Leu Phe Asp Leu
1 5 10 15

<210> SEQ ID NO 27
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: CDR domain from phage library

<400> SEQUENCE: 27

Phe Gln Tyr Gly Ser Ile Pro Pro
1 5

<210> SEQ ID NO 28
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: CDR domain from phage library

<400> SEQUENCE: 28

Ser Met Asn Ser Thr Met Tyr Trp Tyr Leu Arg Arg Val Leu Phe Asp
1 5 10 15

His

<210> SEQ ID NO 29
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: CDR domain from phage library

<400> SEQUENCE: 29

Gln Ser Tyr Asp Met Tyr Met Tyr Ile
1 5

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<210> SEQ ID NO 30
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: polynucleotide sequence of CDR domain from
phage library

<400> SEQUENCE: 30
gattttcttg gttatgagtt tgattat 27

<210> SEQ ID NO 31
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: polynucleotide sequence of CDR domain from
phage library

<400> SEQUENCE: 31
cagagctatg actattctgc tgattat 27

<210> SEQ ID NO 32
<211> LENGTH: 51
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: polynucleotide sequence of CDR domain from
phage library

<400> SEQUENCE: 32
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<210> SEQ ID NO 33
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: polynucleotide sequence of CDR domain from
phage library

<400> SEQUENCE: 33
cagtctcatc atttttatga g 21

<210> SEQ ID NO 34
<211> LENGTH: 60
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: polynucleotide sequence of CDR domain from
phage library

<400> SEQUENCE: 34
tatcattctt ggtatgagat ggggttattat ggttctactg ttggttatat gtttgattat 60

<210> SEQ ID NO 35
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: polynucleotide sequence of CDR domain from
phage library

<400> SEQUENCE: 35

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cagagctatg actttgattt tgct 24

<210> SEQ ID NO 36
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: polynucleotide sequence of CDR domain from
phage library

<400> SEQUENCE: 36

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<210> SEQ ID NO 37
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: polynucleotide sequence of CDR domain from
phage library

<400> SEQUENCE: 37

cagcagtatg attctattcc ttat 24

<210> SEQ ID NO 38
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: polynucleotide sequence of CDR domain from
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<400> SEQUENCE: 38

gttaatcatt ggacttatac ttttgattat 30

<210> SEQ ID NO 39
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: polynucleotide sequence of CDR domain from
phage library

<400> SEQUENCE: 39

cagcagatgt ctaattatcc tgat 24

<210> SEQ ID NO 40
<211> LENGTH: 51
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: polynucleotide sequence of CDR domain from
phage library

<400> SEQUENCE: 40

ggttattggt atgcttattt tacttatatt aattatgggt attttgataa t 51

<210> SEQ ID NO 41
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: polynucleotide sequence of CDR domain from
phage library

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<400> SEQUENCE: 41

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27

<210> SEQ ID NO 42

<211> LENGTH: 45

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: polynucleotide sequence of CDR domain from
phage library

<400> SEQUENCE: 42

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45

<210> SEQ ID NO 43

<211> LENGTH: 24

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: polynucleotide sequence of CDR domain from
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<400> SEQUENCE: 43

tttcagtatg gttctattcc tcct

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<210> SEQ ID NO 44

<211> LENGTH: 54

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: polynucleotide sequence of CDR domain from
phage library

<400> SEQUENCE: 44

acttggcagt attcttattt ttattatctt gatgggtgggtt attattttga tatt

54

<210> SEQ ID NO 45

<211> LENGTH: 24

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: polynucleotide sequence of CDR domain from
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<400> SEQUENCE: 45

cagcagacta ataatgctcc tggt

24

<210> SEQ ID NO 46

<211> LENGTH: 51

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: polynucleotide sequence of CDR domain from
phage library

<400> SEQUENCE: 46

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51

<210> SEQ ID NO 47

<211> LENGTH: 24

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

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<223> OTHER INFORMATION: polynucleotide sequence of CDR domain from phage library

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<210> SEQ ID NO 48

<211> LENGTH: 51

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: polynucleotide sequence of CDR domain from phage library

<400> SEQUENCE: 48

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<210> SEQ ID NO 49

<211> LENGTH: 27

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: polynucleotide sequence of CDR domain from phage library

<400> SEQUENCE: 49

cagagctatg acatgtataa ttatatt 27

<210> SEQ ID NO 50

<211> LENGTH: 24

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: polynucleotide sequence of CDR domain from phage library

<400> SEQUENCE: 50

tcttattatc ctgattttga ttat 24

<210> SEQ ID NO 51

<211> LENGTH: 27

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: polynucleotide sequence of CDR domain from phage library

<400> SEQUENCE: 51

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<210> SEQ ID NO 52

<211> LENGTH: 5020

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: polynucleotide sequence of cloning vector

<300> PUBLICATION INFORMATION:

<301> AUTHORS: Knappik et al

<302> TITLE: Fully synthetic human combinatorial antibody libraries (HuCAL) based on modular consensus frameworks and CDRs randomized with trinucleotides.

<303> JOURNAL: J Mol Biol

<304> VOLUME: 296

<305> ISSUE: 1

<306> PAGES: 57-86

<307> DATE: 2000-02-11

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<308> DATABASE ACCESSION NUMBER: pubmed/10656818

<309> DATABASE ENTRY DATE: 2000-02-11

<313> RELEVANT RESIDUES: (1)..(5020)

<400> SEQUENCE: 52

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caccacacc gccgcgctta atgcgcgctt acagggcgcg tgctagacta gtgtttaaac      2040
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ggcggaagaa gttgtccata ttggctacgt ttaaatcaaa actggtgaaa ctcaccacgg	4260
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<210> SEQ ID NO 53
<211> LENGTH: 4151
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: polynucleotide sequence of cloning vector
<300> PUBLICATION INFORMATION:
<301> AUTHORS: knappik et al
<302> TITLE: Fully synthetic human combinatorial antibody libraries
(HuCAL) based on modular consensus frameworks and CDRs randomized
with trinucleotides.
<303> JOURNAL: j mol biol
<304> VOLUME: 296
<305> ISSUE: 1
<306> PAGES: 57-86
<307> DATE: 2000-02-11
<308> DATABASE ACCESSION NUMBER: pubmed/10656818
<309> DATABASE ENTRY DATE: 2000-02-11
<313> RELEVANT RESIDUES: (1)..(4151)

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<400> SEQUENCE: 53

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agcagccgtg caactgggggt cccggcgcggt tttagcggct ctggatccgg cacggatttt 300
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tctaattgta gttagctcac tcattaggca cccaggtctt tacactttat gcttccggct 4080
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gattacgaat t 4151

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<210> SEQ ID NO 54
<211> LENGTH: 306
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: polynucleotide sequence of a VL domain
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (253)..(255)
<223> OTHER INFORMATION: NNN=ACT OR GTT

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<400> SEQUENCE: 54

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attacctgca gagcgagcca gggcattagc agctatctgg cgtggtacca gcagaaacca 120
ggtaaagcac cgaaactatt aatttatgca gccagcagct tgcaaagcgg ggtcccgtcc 180
cgtttttagcg gctctggatc cggcactgat tttaccctga ccattagcag cctgcaacct 240
gaagactttg cgnnntatta ttgccagacc tttggccagg gtacgaaagt tgaattaaa 300
cgtacg 306

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<210> SEQ ID NO 55
<211> LENGTH: 327
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: polynucleotide sequence of a VL domain

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<400> SEQUENCE: 55

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attacctgca gagcgagcca gggcattagc agctatctgg cgtggtacca gcagaaacca 120
ggtaaagcac cgaaactatt aatttatgca gccagcagct tgcaaagcgg ggtcccgtcc 180
cgtttttagcg gctctggatc cggcactgat tttaccctga ccattagcag cctgcaacct 240

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gaagactttg cggtttatta ttgctttcag tatggttcta ttcctcctac ctttgccag 300
ggtacgaaag ttgaaattaa acgtacg 327

<210> SEQ ID NO 56
<211> LENGTH: 309
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: polynucleotide sequence of a VL domain
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (256)..(258)
<223> OTHER INFORMATION: NNN=ACT OR GTT

<400> SEQUENCE: 56

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ccagggtcaag caccgcgtct attaatattat ggcgcgagca gccgtgcaac tgggggtccc 180
gcgcgtttta gcggctctgg atccggcacg gatatttacc tgaccattag cagcctggaa 240
cctgaagact ttgcgnnta ttattgccag acctttggcc agggtagcaa agttgaaatt 300
aaacgtacg 309

<210> SEQ ID NO 57
<211> LENGTH: 330
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: polynucleotide sequence of a VL domain

<400> SEQUENCE: 57

gatatcgtgc tgaccagag cccggcgacc ctgagcctgt ctccgggcca acgtgcgacc 60
ctgagctgca gagcgagcca gagcgtgagc agcagctatc tggcgtggta ccagcagaaa 120
ccagggtcaag caccgcgtct attaatattat ggcgcgagca gccgtgcaac tgggggtccc 180
gcgcgtttta gcggctctgg atccggcacg gatatttacc tgaccattag cagcctggaa 240
cctgaagact ttgcgactta ttattgccag cagatgtcta attatcctga tacctttggc 300
cagggtacga aagttgaaat taaacgtacg 330

<210> SEQ ID NO 58
<211> LENGTH: 330
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: polynucleotide sequence of a VL domain

<400> SEQUENCE: 58

gatatcgtgc tgaccagag cccggcgacc ctgagcctgt ctccgggcca acgtgcgacc 60
ctgagctgca gagcgagcca gagcgtgagc agcagctatc tggcgtggta ccagcagaaa 120
ccagggtcaag caccgcgtct attaatattat ggcgcgagca gccgtgcaac tgggggtccc 180
gcgcgtttta gcggctctgg atccggcacg gatatttacc tgaccattag cagcctggaa 240
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cagggtacga aagttgaaat taaacgtacg 330

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<210> SEQ ID NO 59
<211> LENGTH: 324
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: polynucleotide sequence of a VL domain

<400> SEQUENCE: 59

gatatcgtga tgacccagag cccggatagc ctggcgggtga gcctggggcga acgtgcgacc 60
attaactgca gaagcagcca gagcgtgctg tatagcagca acaacaaaaa ctatctggcg 120
tggtaccagc agaaaccagg tcagccgccc aaactattaa tttattgggc atccaccctg 180
gaaagcgggg tcccggatcg ttttagcggc tctggatccg gcaactgattt taccctgacc 240
atttcgtccc tgcaagctga agacgtggcg gtgtattatt gccagacctt tggccagggt 300
acgaaagtgt aaattaaacg tacg 324

<210> SEQ ID NO 60
<211> LENGTH: 345
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: polynucleotide sequence of a VL domain

<400> SEQUENCE: 60

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attaactgca gaagcagcca gagcgtgctg tatagcagca acaacaaaaa ctatctggcg 120
tggtaccagc agaaaccagg tcagccgccc aaactattaa tttattgggc atccaccctg 180
gaaagcgggg tcccggatcg ttttagcggc tctggatccg gcaactgattt taccctgacc 240
atttcgtccc tgcaagctga agacgtggcg gtgtattatt gccagcagta tgattctatt 300
ccttatacct ttggccaggg tacgaaagt gaaattaaac gtacg 345

<210> SEQ ID NO 61
<211> LENGTH: 315
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: polynucleotide sequence of a VL domain

<400> SEQUENCE: 61

gatatcgcac tgacccagcc agcttcagt agcggctcac caggtcagag cattaccatc 60
tcgtgtacgg gtactagcag cgatgtgggc ggctataact atgtgagctg gtaccagcag 120
catcccggga aggcgcccga actgatgatt tatgatgtga gcaaccgtcc ctccaggcgtg 180
agcaaccgtt ttagcggatc caaaagcggc aacaccgcga gcctgaccat tagcggcctg 240
caagcggaag acgaagcgga ttattattgc caggacgtgt ttggcggcgg cacgaagtta 300
accgttcttg gccag 315

<210> SEQ ID NO 62
<211> LENGTH: 336
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: polynucleotide sequence of a VL domain

<400> SEQUENCE: 62

gatatcgcac tgacccagcc agcttcagt agcggctcac caggtcagag cattaccatc 60

-continued

```

tcgtgtacgg gtactagcag cgatgtgggc ggctataact atgtgagctg gtaccagcag 120
catccccgga aggcgccgaa actgatgatt tatgatgtga gcaaccgtcc ctccaggcgtg 180
agcaaccggt ttagcggatc caaaagcggc aacaccgcga gcctgacat tagcggcctg 240
caagcggaag acgaagcgga ttattattgc cagagctatg acatgtataa ttattattgtg 300
tttggcggcg gcacgaagtt aaccgttctt ggccag 336

```

```

<210> SEQ ID NO 63
<211> LENGTH: 330
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: polynucleotide sequence of a VL domain

```

```

<400> SEQUENCE: 63

```

```

gatatcgac tgaccagcc agcttcagtg agcggctcac caggtcagag cattaccatc 60
tcgtgtacgg gtactagcag cgatgtgggc ggctataact atgtgagctg gtaccagcag 120
catccccgga aggcgccgaa actgatgatt tatgatgtga gcaaccgtcc ctccaggcgtg 180
agcaaccggt ttagcggatc caaaagcggc aacaccgcga gcctgacat tagcggcctg 240
caagcggaag acgaagcgga ttattattgc cagtctcatc atttttatga ggtgtttggc 300
ggcggcacga agttaaccgt tcttggccag 330

```

```

<210> SEQ ID NO 64
<211> LENGTH: 336
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: polynucleotide sequence of a VL domain

```

```

<400> SEQUENCE: 64

```

```

gatatcgac tgaccagcc agcttcagtg agcggctcac caggtcagag cattaccatc 60
tcgtgtacgg gtactagcag cgatgtgggc ggctataact atgtgagctg gtaccagcag 120
catccccgga aggcgccgaa actgatgatt tatgatgtga gcaaccgtcc ctccaggcgtg 180
agcaaccggt ttagcggatc caaaagcggc aacaccgcga gcctgacat tagcggcctg 240
caagcggaag acgaagcgga ttattattgc cagagctatg acaataattc tgatgttgtg 300
tttggcggcg gcacgaagtt aaccgttctt ggccag 336

```

```

<210> SEQ ID NO 65
<211> LENGTH: 306
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: polynucleotide sequence of a VL domain

```

```

<400> SEQUENCE: 65

```

```

gatatcgaac tgaccagcc gccttcagtg agcgttgac caggtcagac cgcgcgtatc 60
tcgtgtacgg gcgatgcgct gggcgataaa tacgcgagct ggtaccagca gaaaccggg 120
caggcgccag ttctggtgat ttatgatgat tctgaccgtc cctcaggcat cccggaacgc 180
tttagcggat ccaacagcgg caacaccgcg accctgacca ttagcggcac tcaggcgga 240
gacgaagcgg attattattg ccaggacgtg tttggcggcg gcacgaagtt aaccgttctt 300
ggccag 306

```

-continued

<210> SEQ ID NO 66
<211> LENGTH: 324
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: polynucleotide sequence of a VL domain

<400> SEQUENCE: 66

```
gatatcgaac tgacccagcc gccttcagtg agcgttgac caggtcagac cgcgcgtatc    60
tcgtgtagcg gcgatgcgct gggcgataaa tacgcgagct ggtaccagca gaaacccggg    120
caggcgccag ttctggtgat ttatgatgat tctgaccgtc cctcaggcat cccggaacgc    180
tttagcggat ccaacagcgg caacaccgcg accctgacca ttagcggcac tcaggcgga    240
gacgaagcgg attattattg ccagagctat gactatttta agcttggtgtt tggcgcgggc    300
acgaagttaa ccgttcttgg ccag                                           324
```

<210> SEQ ID NO 67
<211> LENGTH: 327
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: polynucleotide sequence of a VL domain

<400> SEQUENCE: 67

```
gatatcgaac tgacccagcc gccttcagtg agcgttgac caggtcagac cgcgcgtatc    60
tcgtgtagcg gcgatgcgct gggcgataaa tacgcgagct ggtaccagca gaaacccggg    120
caggcgccag ttctggtgat ttatgatgat tctgaccgtc cctcaggcat cccggaacgc    180
tttagcggat ccaacagcgg caacaccgcg accctgacca ttagcggcac tcaggcgga    240
gacgaagcgg attattattg ccagagctat gactattctg ctgattatgt gtttggcggc    300
ggcacgaagt taaccgttct tggccag                                           327
```

<210> SEQ ID NO 68
<211> LENGTH: 324
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: polynucleotide sequence of a VL domain

<400> SEQUENCE: 68

```
gatatcgaac tgacccagcc gccttcagtg agcgttgac caggtcagac cgcgcgtatc    60
tcgtgtagcg gcgatgcgct gggcgataaa tacgcgagct ggtaccagca gaaacccggg    120
caggcgccag ttctggtgat ttatgatgat tctgaccgtc cctcaggcat cccggaacgc    180
tttagcggat ccaacagcgg caacaccgcg accctgacca ttagcggcac tcaggcgga    240
gacgaagcgg attattattg ccagagctat gactttgatt ttgctgtgtt tggcgcgggc    300
acgaagttaa ccgttcttgg ccag                                           324
```

<210> SEQ ID NO 69
<211> LENGTH: 327
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: polynucleotide sequence of a VL domain

<400> SEQUENCE: 69

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```

gatatcgaac tgacccagcc gccttcagtg agcggtgcac caggtcagac cgcgcgtatc      60
tcgtgtagcg gcgatgcgct gggcgataaa tacgcgagct ggtaccagca gaaacccggg    120
caggcgccag ttctggtgat ttatgatgat tctgaccgtc cctcaggcat cccggaacgc    180
tttagcggat ccaacagcgg caacaccgcg accctgacca ttagcggcac tcaggcggaa    240
gacgaagcgg attattattg ccagagctat gacggtcctg atctttgggt gtttgccggc    300
ggcacgaagt taaccgttct tggccag                                           327

```

```

<210> SEQ ID NO 70
<211> LENGTH: 332
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: polynucleotide sequence of a VH domain
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(3)
<223> OTHER INFORMATION: NNN=GAA OR CAG

```

```

<400> SEQUENCE: 70
nnngtgcaat tggttcagtc tggcgcgga gtaaaaaaac cgggcagcag cgtgaaagtg      60
agctgcaaa g cctccggagg cacttttagc agctatgcga ttagctgggt gcgccaagcc    120
cctgggcagg gtctcgagtg gatgggcggc attattccga tttttggcac ggcgaactac    180
gcgcagaagt ttcagggcgg ggtgaccatt accgcggatg aaagcaccag caccgcgtat    240
atggaactga gcagcctgcg tagcgaagat acggccgtgt attattgcgc gcgtgattgg    300
ggccaaggca ccctggtgac ggttagctca gc                                           332

```

```

<210> SEQ ID NO 71
<211> LENGTH: 359
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: polynucleotide sequence of a VH domain

```

```

<400> SEQUENCE: 71
cagggtgcaat tggttcagtc tggcgcgga gtaaaaaaac cgggcagcag cgtgaaagtg      60
agctgcaaa g cctccggagg cacttttagc agctatgcga ttagctgggt gcgccaagcc    120
cctgggcagg gtctcgagtg gatgggcggc attattccga tttttggcac ggcgaactac    180
gcgcagaagt ttcagggcgg ggtgaccatt accgcggatg aaagcaccag caccgcgtat    240
atggaactga gcagcctgcg tagcgaagat acggccgtgt attattgcgc gcgtgataat    300
tggtttaagc ctttttctga tgtttggggc caaggcacc cttgtgacgg tagctcagc      359

```

```

<210> SEQ ID NO 72
<211> LENGTH: 359
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: polynucleotide sequence of a VH domain

```

```

<400> SEQUENCE: 72
cagggtgcaat tggttcagtc tggcgcgga gtaaaaaaac cgggcagcag cgtgaaagtg      60
agctgcaaa g cctccggagg cacttttagc agctatgcga ttagctgggt gcgccaagcc    120
cctgggcagg gtctcgagtg gatgggcggc attattccga tttttggcac ggcgaactac    180

```

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```

gcgcagaagt ttcagggccg ggtgaccatt accgcggatg aaagcaccag caccgcgtat 240
atggaactga gcagcctgcg tagcgaagat acggccgtgt attattgcgc gcgtgttaat 300
cattggactt atacttttga ttattggggc caaggcacc c ttgtgacggt tagctcagc 359

```

```

<210> SEQ ID NO 73
<211> LENGTH: 374
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: polynucleotide sequence of a VH domain

```

```

<400> SEQUENCE: 73

```

```

caggtgcaat tggttcagtc tggcgcgga gtgaaaaaac cgggcagcag cgtgaaagt 60
agctgcaaa cctccggagg cacttttagc agctatgcga ttagctgggt gcgccaagcc 120
cctgggcagg gtctcagtg gatggggcgc attattccga tttttggcac ggcgaactac 180
gcgcagaagt ttcagggccg ggtgaccatt accgcggatg aaagcaccag caccgcgtat 240
atggaactga gcagcctgcg tagcgaagat acggccgtgt attattgcgc gcgtggtggt 300
ggttgggttt ctcatggtta ttattatctt ttgatcttt ggggccaagg caccctggtg 360
acggttagct cagc 374

```

```

<210> SEQ ID NO 74
<211> LENGTH: 332
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: polynucleotide sequence of a VH domain
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(3)
<223> OTHER INFORMATION: NNN=GAA OR CAG

```

```

<400> SEQUENCE: 74

```

```

nnngtgcaat tggttcagag cggcgcgga gtgaaaaaac cgggcgcgag cgtgaaagt 60
agctgcaaa cctccggata tacctttacc agctattata tgcaactgggt ccgccaagcc 120
cctgggcagg gtctcagtg gatgggctgg attaacccga atagcggcgg cacgaactac 180
gcgcagaagt ttcagggccg ggtgaccatg acccgtgata ccagcattag caccgcgtat 240
atggaactga gcagcctgcg tagcgaagat acggccgtgt attattgcgc gcgtgattgg 300
ggccaaggca ccctggtgac ggttagctca gc 332

```

```

<210> SEQ ID NO 75
<211> LENGTH: 380
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: polynucleotide sequence of a VH domain

```

```

<400> SEQUENCE: 75

```

```

caggtgcaat tggttcagag cggcgcgga gtgaaaaaac cgggcgcgag cgtgaaagt 60
agctgcaaa cctccggata tacctttacc agctattata tgcaactgggt ccgccaagcc 120
cctgggcagg gtctcagtg gatgggctgg attaacccga atagcggcgg cacgaactac 180
gcgcagaagt ttcagggccg ggtgaccatg acccgtgata ccagcattag caccgcgtat 240
atggaactga gcagcctgcg tagcgaagat acggccgtgt attattgcgc gcgtaatatg 300

```

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```
gcttatacta attatcagta tgtaatatg cctcattttg attattgggg ccaaggcacc 360
ctgggtgacgg ttagctcagc 380
```

```
<210> SEQ ID NO 76
<211> LENGTH: 380
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: polynucleotide sequence of a VH domain
```

```
<400> SEQUENCE: 76
```

```
cagggtgcaat tgggttcagag cggcgcgga gtgaaaaaac cggcgcgag cgtgaaagtg 60
agctgcaaag cctccggata tacctttacc agctattata tgcactgggt ccgccaagcc 120
cctgggcagg gtctcgagt gatgggctgg attaaccca atagcggcg caggaactac 180
gcgcagaagt ttcagggccg ggtgaccatg acccgtgata ccagcattag caccgcgtat 240
atggaactga gcagcctgcg tagcgaagat acggccgtgt attattgcgc gcgttctatg 300
aatttacta tgatttgta tcttcgtcgt gttctttttg atcattgggg ccaaggcacc 360
ctgggtgacgg ttagctcagc 380
```

```
<210> SEQ ID NO 77
<211> LENGTH: 356
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: polynucleotide sequence of a VH domain
```

```
<400> SEQUENCE: 77
```

```
cagggtgcaat tgggttcagag cggcgcgga gtgaaaaaac cggcgcgag cgtgaaagtg 60
agctgcaaag cctccggata tacctttacc agctattata tgcactgggt ccgccaagcc 120
cctgggcagg gtctcgagt gatgggctgg attaaccca atagcggcg caggaactac 180
gcgcagaagt ttcagggccg ggtgaccatg acccgtgata ccagcattag caccgcgtat 240
atggaactga gcagcctgcg tagcgaagat acggccgtgt attattgcgc gcgtgatattt 300
cttggttatg agtttgatta ttggggccaa ggcaccctgg tgacggttag ctacgc 356
```

```
<210> SEQ ID NO 78
<211> LENGTH: 380
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: polynucleotide sequence of a VH domain
```

```
<400> SEQUENCE: 78
```

```
cagggtgcaat tgggttcagag cggcgcgga gtgaaaaaac cggcgcgag cgtgaaagtg 60
agctgcaaag cctccggata tacctttacc agctattata tgcactgggt ccgccaagcc 120
cctgggcagg gtctcgagt gatgggctgg attaaccca atagcggcg caggaactac 180
gcgcagaagt ttcagggccg ggtgaccatg acccgtgata ccagcattag caccgcgtat 240
atggaactga gcagcctgcg tagcgaagat acggccgtgt attattgcgc gcgttattat 300
ggttcttctc ttatcatta tgtttttggt ggttttattg attattgggg ccaaggcacc 360
ctgggtgacgg ttagctcagc 380
```

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<210> SEQ ID NO 79
<211> LENGTH: 380
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: polynucleotide sequence of a VH domain

<400> SEQUENCE: 79

cagggtgcaat tgggttcagag cggcgccggaa gtgaaaaaac cgggcgcgag cgtgaaagtg 60
agctgcaaag cctccggata tacctttacc agctattata tgcactgggt ccgccaagcc 120
cctgggcagg gtctcgatg gatgggctgg attaacccga atagcggcgg cacgaactac 180
gcgcagaagt ttcagggccg ggtgaccatg acccgtgata ccagcattag caccgcgtat 240
atggaactga gcagcctgcg tagcgaagat acggccctgt attattgcgc gcgtggttat 300
tggtagctt attttactta tattaattat gggtattttg ataattgggg ccaaggcacc 360
ctggtagcgg ttagctcagc 380

<210> SEQ ID NO 80
<211> LENGTH: 383
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: polynucleotide sequence of a VH domain

<400> SEQUENCE: 80

cagggtgcaat tgggttcagag cggcgccggaa gtgaaaaaac cgggcgcgag cgtgaaagtg 60
agctgcaaag cctccggata tacctttacc agctattata tgcactgggt ccgccaagcc 120
cctgggcagg gtctcgatg gatgggctgg attaacccga atagcggcgg cacgaactac 180
gcgcagaagt ttcagggccg ggtgaccatg acccgtgata ccagcattag caccgcgtat 240
atggaactga gcagcctgcg tagcgaagat acggccctgt attattgcgc gcgtacttgg 300
cagtattctt atttttatta tcttgatggt gggtattatt ttgatatttg gggccaaggc 360
accctggtga cggtagctc agc 383

<210> SEQ ID NO 81
<211> LENGTH: 335
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: polynucleotide sequence of a VH domain
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(3)
<223> OTHER INFORMATION: NNN=GAA OR CAG

<400> SEQUENCE: 81

nnngtgcaat tgaaagaaag cggcccgcc ctggtgaaac cgacccaaac cctgaccctg 60
acctgtacct tttccgatt tagcctgtcc acgtctggcg ttggcgtggg ctggattcgc 120
cagccgcctg ggaaagccct cgagtggctg gctctgattg attgggatga tgataagtat 180
tatagacca gcctgaaaac gcgtctgacc attagcaaag atacttcgaa aaatcagggtg 240
gtgtgacta tgaccaacat ggaccgggtg gatacggcca cctattattg gcgcgctgat 300
tggggccaag gcaccctggt gacggtagc tcagc 335

<210> SEQ ID NO 82
<211> LENGTH: 392

-continued

<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: polynucleotide sequence of a VH domain

<400> SEQUENCE: 82
cagggtgcaat tgaaagaaag cggcccggcc ctggtgaaac cgacccaaac cctgaccctg 60
acctgtacct tttccggatt tagcctgtcc acgtctggcg ttggcgtggg ctggattcgc 120
cagccgcctg ggaaagccct cgagtggctg gctctgattg attgggatga tgataagtat 180
tatagcacca gcctgaaaac gcgtctgacc attagcaaag atacttcgaa aaatcagggtg 240
gtgtctgacta tgaccaacat ggaccgggtg gatacggcca cctattattg cgcgcgttat 300
cattcttggt atgagatggg ttattatggt tctactgttg gttatatggt tgattattgg 360
ggccaaggca ccctggtgac ggtagctca gc 392

<210> SEQ ID NO 83
<211> LENGTH: 341
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: polynucleotide sequence of a VH domain
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(3)
<223> OTHER INFORMATION: NNN=GAA OR CAG

<400> SEQUENCE: 83
nnngtgcaat tgcaacagtc tggtcgggc ctggtgaaac cgagccaaac cctgagcctg 60
acctgtgcga tttccggaga tagcgtgagc agcaacagcg cggcgtggaa ctggattcgc 120
cagtctcctg ggcgtggcct cgagtggctg ggccgtacct attatcgtag caaatggtat 180
aacgattatg cggtagcgt gaaaagccg attaccatca acccggtac ttcgaaaaac 240
cagtttagcc tgcaactgaa cagcgtgacc ccggaagata cggccgtgta ttattgcgcg 300
cgtgattggg gcccaaggcac cctggtgacg gttagctcag c 341

<210> SEQ ID NO 84
<211> LENGTH: 362
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: polynucleotide sequence of a VH domain

<400> SEQUENCE: 84
cagggtgcaat tgcaacagtc tggtcgggc ctggtgaaac cgagccaaac cctgagcctg 60
acctgtgcga tttccggaga tagcgtgagc agcaacagcg cggcgtggaa ctggattcgc 120
cagtctcctg ggcgtggcct cgagtggctg ggccgtacct attatcgtag caaatggtat 180
aacgattatg cggtagcgt gaaaagccg attaccatca acccggtac ttcgaaaaac 240
cagtttagcc tgcaactgaa cagcgtgacc ccggaagata cggccgtgta ttattgcgcg 300
cgttcttatt atcctgattt tgattattgg ggccaaggca ccctggtgac ggtagctca 360
gc 362

<210> SEQ ID NO 85
<211> LENGTH: 109
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence

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<220> FEATURE:
<223> OTHER INFORMATION: polypeptide sequence of a VL domain

<400> SEQUENCE: 85
Asp Ile Glu Leu Thr Gln Pro Pro Ser Val Ser Val Ala Pro Gly Gln
1 5 10 15
Thr Ala Arg Ile Ser Cys Ser Gly Asp Ala Leu Gly Asp Lys Tyr Ala
20 25 30
Ser Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val Ile Tyr
35 40 45
Asp Asp Ser Asp Arg Pro Ser Gly Ile Pro Glu Arg Phe Ser Gly Ser
50 55 60
Asn Ser Gly Asn Thr Ala Thr Leu Thr Ile Ser Gly Thr Gln Ala Glu
65 70 75 80
Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Tyr Asp Tyr Ser Ala Asp Tyr
85 90 95
Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Gln
100 105

<210> SEQ ID NO 86
<211> LENGTH: 110
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: polypeptide sequence of a VL domain

<400> SEQUENCE: 86
Asp Ile Ala Leu Thr Gln Pro Ala Ser Val Ser Gly Ser Pro Gly Gln
1 5 10 15
Ser Ile Thr Ile Ser Cys Thr Gly Thr Ser Ser Asp Val Gly Gly Tyr
20 25 30
Asn Tyr Val Ser Trp Tyr Gln Gln His Pro Gly Lys Ala Pro Lys Leu
35 40 45
Met Ile Tyr Asp Val Ser Asn Arg Pro Ser Gly Val Ser Asn Arg Phe
50 55 60
Ser Gly Ser Lys Ser Gly Asn Thr Ala Ser Leu Thr Ile Ser Gly Leu
65 70 75 80
Gln Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser His His Phe Tyr
85 90 95
Glu Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Gln
100 105 110

<210> SEQ ID NO 87
<211> LENGTH: 108
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: polypeptide sequence of a VL domain

<400> SEQUENCE: 87
Asp Ile Glu Leu Thr Gln Pro Pro Ser Val Ser Val Ala Pro Gly Gln
1 5 10 15
Thr Ala Arg Ile Ser Cys Ser Gly Asp Ala Leu Gly Asp Lys Tyr Ala
20 25 30
Ser Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val Ile Tyr
35 40 45

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Asp Asp Ser Asp Arg Pro Ser Gly Ile Pro Glu Arg Phe Ser Gly Ser
 50          55          60
Asn Ser Gly Asn Thr Ala Thr Leu Thr Ile Ser Gly Thr Gln Ala Glu
65          70          75          80
Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Tyr Asp Phe Asp Phe Ala Val
          85          90          95
Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Gln
      100          105

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<210> SEQ ID NO 88
<211> LENGTH: 115
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: polypeptide sequence of a VL domain

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<400> SEQUENCE: 88

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Asp Ile Val Met Thr Gln Ser Pro Asp Ser Leu Ala Val Ser Leu Gly
1          5          10          15
Glu Arg Ala Thr Ile Asn Cys Arg Ser Ser Gln Ser Val Leu Tyr Ser
          20          25          30
Ser Asn Asn Lys Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln
          35          40          45
Pro Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val
          50          55          60
Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
65          70          75          80
Ile Ser Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln
          85          90          95
Tyr Asp Ser Ile Pro Tyr Thr Phe Gly Gln Gly Thr Lys Val Glu Ile
          100          105          110
Lys Arg Thr
      115

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<210> SEQ ID NO 89
<211> LENGTH: 110
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: polypeptide sequence of a VL domain

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<400> SEQUENCE: 89

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Asp Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
1          5          10          15
Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Ser
          20          25          30
Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
          35          40          45
Ile Tyr Gly Ala Ser Ser Arg Ala Thr Gly Val Pro Ala Arg Phe Ser
          50          55          60
Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu
65          70          75          80
Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Met Ser Asn Tyr Pro
          85          90          95
Asp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr
          100          105          110

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-continued

<210> SEQ ID NO 90
<211> LENGTH: 112
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: polypeptide sequence of a VL domain

<400> SEQUENCE: 90

Asp Ile Ala Leu Thr Gln Pro Ala Ser Val Ser Gly Ser Pro Gly Gln
1 5 10 15
Ser Ile Thr Ile Ser Cys Thr Gly Thr Ser Ser Asp Val Gly Gly Tyr
20 25 30
Asn Tyr Val Ser Trp Tyr Gln Gln His Pro Gly Lys Ala Pro Lys Leu
35 40 45
Met Ile Tyr Asp Val Ser Asn Arg Pro Ser Gly Val Ser Asn Arg Phe
50 55 60
Ser Gly Ser Lys Ser Gly Asn Thr Ala Ser Leu Thr Ile Ser Gly Leu
65 70 75 80
Gln Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Tyr Asp Asn Asn
85 90 95
Ser Asp Val Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Gln
100 105 110

<210> SEQ ID NO 91
<211> LENGTH: 109
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: polypeptide sequence of a VL domain

<400> SEQUENCE: 91

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Tyr
20 25 30
Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45
Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80
Glu Asp Phe Ala Val Tyr Tyr Cys Phe Gln Tyr Gly Ser Ile Pro Pro
85 90 95
Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr
100 105

<210> SEQ ID NO 92
<211> LENGTH: 110
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: polypeptide sequence of a VL domain

<400> SEQUENCE: 92

Asp Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
1 5 10 15

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Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Ser
 20 25 30
 Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
 35 40 45
 Ile Tyr Gly Ala Ser Ser Arg Ala Thr Gly Val Pro Ala Arg Phe Ser
 50 55 60
 Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu
 65 70 75 80
 Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Thr Asn Asn Ala Pro
 85 90 95
 Val Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr
 100 105 110

<210> SEQ ID NO 93
 <211> LENGTH: 108
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: polypeptide sequence of a VL domain

<400> SEQUENCE: 93

Asp Ile Glu Leu Thr Gln Pro Pro Ser Val Ser Val Ala Pro Gly Gln
 1 5 10 15
 Thr Ala Arg Ile Ser Cys Ser Gly Asp Ala Leu Gly Asp Lys Tyr Ala
 20 25 30
 Ser Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val Ile Tyr
 35 40 45
 Asp Asp Ser Asp Arg Pro Ser Gly Ile Pro Glu Arg Phe Ser Gly Ser
 50 55 60
 Asn Ser Gly Asn Thr Ala Thr Leu Thr Ile Ser Gly Thr Gln Ala Glu
 65 70 75 80
 Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Tyr Asp Tyr Phe Lys Leu Val
 85 90 95
 Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Gln
 100 105

<210> SEQ ID NO 94
 <211> LENGTH: 112
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: polypeptide sequence of a VL domain

<400> SEQUENCE: 94

Asp Ile Ala Leu Thr Gln Pro Ala Ser Val Ser Gly Ser Pro Gly Gln
 1 5 10 15
 Ser Ile Thr Ile Ser Cys Thr Gly Thr Ser Ser Asp Val Gly Gly Tyr
 20 25 30
 Asn Tyr Val Ser Trp Tyr Gln Gln His Pro Gly Lys Ala Pro Lys Leu
 35 40 45
 Met Ile Tyr Asp Val Ser Asn Arg Pro Ser Gly Val Ser Asn Arg Phe
 50 55 60
 Ser Gly Ser Lys Ser Gly Asn Thr Ala Ser Leu Thr Ile Ser Gly Leu
 65 70 75 80
 Gln Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Tyr Asp Met Tyr
 85 90 95

-continued

Asn Tyr Ile Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Gln
100 105 110

<210> SEQ ID NO 95
<211> LENGTH: 109
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: polypeptide sequence of a VL domain

<400> SEQUENCE: 95

Asp Ile Glu Leu Thr Gln Pro Pro Ser Val Ser Val Ala Pro Gly Gln
1 5 10 15

Thr Ala Arg Ile Ser Cys Ser Gly Asp Ala Leu Gly Asp Lys Tyr Ala
20 25 30

Ser Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val Ile Tyr
35 40 45

Asp Asp Ser Asp Arg Pro Ser Gly Ile Pro Glu Arg Phe Ser Gly Ser
50 55 60

Asn Ser Gly Asn Thr Ala Thr Leu Thr Ile Ser Gly Thr Gln Ala Glu
65 70 75 80

Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Tyr Asp Gly Pro Asp Leu Trp
85 90 95

Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Gln
100 105

<210> SEQ ID NO 96
<211> LENGTH: 118
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: polypeptide sequence of a VH domain

<400> SEQUENCE: 96

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
20 25 30

Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Trp Ile Asn Pro Asn Ser Gly Gly Thr Asn Tyr Ala Gln Lys Phe
50 55 60

Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Ile Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Asp Phe Leu Gly Tyr Glu Phe Asp Tyr Trp Gly Gln Gly Thr
100 105 110

Leu Val Thr Val Ser Ser
115

<210> SEQ ID NO 97
<211> LENGTH: 126
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: polypeptide sequence of a VH domain

-continued

<400> SEQUENCE: 97

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
 1 5 10 15
 Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
 20 25 30
 Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
 35 40 45
 Gly Trp Ile Asn Pro Asn Ser Gly Gly Thr Asn Tyr Ala Gln Lys Phe
 50 55 60
 Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Ile Ser Thr Ala Tyr
 65 70 75 80
 Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95
 Ala Arg Tyr Tyr Gly Ser Ser Leu Tyr His Tyr Val Phe Gly Gly Phe
 100 105 110
 Ile Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
 115 120 125

<210> SEQ ID NO 98

<211> LENGTH: 130

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: polypeptide sequence of a VH domain

<400> SEQUENCE: 98

Gln Val Gln Leu Lys Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln
 1 5 10 15
 Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ser
 20 25 30
 Gly Val Gly Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu
 35 40 45
 Trp Leu Ala Leu Ile Asp Trp Asp Asp Asp Lys Tyr Tyr Ser Thr Ser
 50 55 60
 Leu Lys Thr Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val
 65 70 75 80
 Val Leu Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr
 85 90 95
 Cys Ala Arg Tyr His Ser Trp Tyr Glu Met Gly Tyr Tyr Gly Ser Thr
 100 105 110
 Val Gly Tyr Met Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val
 115 120 125
 Ser Ser
 130

<210> SEQ ID NO 99

<211> LENGTH: 119

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: polypeptide sequence of a VH domain

<400> SEQUENCE: 99

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
 1 5 10 15

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Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Tyr
      20      25      30
Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
      35      40      45
Gly Gly Ile Ile Pro Ile Phe Gly Thr Ala Asn Tyr Ala Gln Lys Phe
      50      55      60
Gln Gly Arg Val Thr Ile Thr Ala Asp Glu Ser Thr Ser Thr Ala Tyr
      65      70      75      80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
      85      90      95
Ala Arg Asp Asn Trp Phe Lys Pro Phe Ser Asp Val Trp Gly Gln Gly
      100      105      110
Thr Leu Val Thr Val Ser Ser
      115

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<210> SEQ ID NO 100
<211> LENGTH: 119
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: polypeptide sequence of a VH domain

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<400> SEQUENCE: 100

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Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1          5          10          15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Tyr
      20      25      30
Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
      35      40      45
Gly Gly Ile Ile Pro Ile Phe Gly Thr Ala Asn Tyr Ala Gln Lys Phe
      50      55      60
Gln Gly Arg Val Thr Ile Thr Ala Asp Glu Ser Thr Ser Thr Ala Tyr
      65      70      75      80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
      85      90      95
Ala Arg Val Asn His Trp Thr Tyr Thr Phe Asp Tyr Trp Gly Gln Gly
      100      105      110
Thr Leu Val Thr Val Ser Ser
      115

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<210> SEQ ID NO 101
<211> LENGTH: 126
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: polypeptide sequence of a VH domain

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<400> SEQUENCE: 101

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Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1          5          10          15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
      20      25      30
Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
      35      40      45
Gly Trp Ile Asn Pro Asn Ser Gly Gly Thr Asn Tyr Ala Gln Lys Phe

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50	55	60
Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Ile Ser Thr Ala Tyr		
65	70	75 80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys		
	85	90 95
Ala Arg Gly Tyr Trp Tyr Ala Tyr Phe Thr Tyr Ile Asn Tyr Gly Tyr		
	100	105 110
Phe Asp Asn Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser		
	115	120 125

<210> SEQ ID NO 102
 <211> LENGTH: 124
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: polypeptide sequence of a VH domain

<400> SEQUENCE: 102

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser		
1	5	10 15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Tyr		
	20	25 30
Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met		
	35	40 45
Gly Gly Ile Ile Pro Ile Phe Gly Thr Ala Asn Tyr Ala Gln Lys Phe		
	50	55 60
Gln Gly Arg Val Thr Ile Thr Ala Asp Glu Ser Thr Ser Thr Ala Tyr		
65	70	75 80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys		
	85	90 95
Ala Arg Gly Gly Gly Trp Val Ser His Gly Tyr Tyr Tyr Leu Phe Asp		
	100	105 110
Leu Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser		
	115	120

<210> SEQ ID NO 103
 <211> LENGTH: 127
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: polypeptide sequence of a VH domain

<400> SEQUENCE: 103

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala		
1	5	10 15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr		
	20	25 30
Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met		
	35	40 45
Gly Trp Ile Asn Pro Asn Ser Gly Gly Thr Asn Tyr Ala Gln Lys Phe		
	50	55 60
Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Ile Ser Thr Ala Tyr		
65	70	75 80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys		
	85	90 95

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Ala Arg Thr Trp Gln Tyr Ser Tyr Phe Tyr Tyr Leu Asp Gly Gly Tyr
100 105 110

Tyr Phe Asp Ile Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120 125

<210> SEQ ID NO 104
 <211> LENGTH: 126
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: polypeptide sequence of a VH domain

<400> SEQUENCE: 104

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
20 25 30

Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Trp Ile Asn Pro Asn Ser Gly Gly Thr Asn Tyr Ala Gln Lys Phe
50 55 60

Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Ile Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Asn Met Ala Tyr Thr Asn Tyr Gln Tyr Val Asn Met Pro His
100 105 110

Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120 125

<210> SEQ ID NO 105
 <211> LENGTH: 126
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: polypeptide sequence of a VH domain

<400> SEQUENCE: 105

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
20 25 30

Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Trp Ile Asn Pro Asn Ser Gly Gly Thr Asn Tyr Ala Gln Lys Phe
50 55 60

Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Ile Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Ser Met Asn Ser Thr Met Tyr Trp Tyr Leu Arg Arg Val Leu
100 105 110

Phe Asp His Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120 125

<210> SEQ ID NO 106

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<211> LENGTH: 120
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: polypeptide sequence of a VH domain

<400> SEQUENCE: 106

Gln Val Gln Leu Gln Gln Ser Gly Pro Gly Leu Val Lys Pro Ser Gln
1             5             10             15

Thr Leu Ser Leu Thr Cys Ala Ile Ser Gly Asp Ser Val Ser Ser Asn
20             25             30

Ser Ala Ala Trp Asn Trp Ile Arg Gln Ser Pro Gly Arg Gly Leu Glu
35             40             45

Trp Leu Gly Arg Thr Tyr Tyr Arg Ser Lys Trp Tyr Asn Asp Tyr Ala
50             55             60

Val Ser Val Lys Ser Arg Ile Thr Ile Asn Pro Asp Thr Ser Lys Asn
65             70             75             80

Gln Phe Ser Leu Gln Leu Asn Ser Val Thr Pro Glu Asp Thr Ala Val
85             90             95

Tyr Tyr Cys Ala Arg Ser Tyr Tyr Pro Asp Phe Asp Tyr Trp Gly Gln
100            105            110

Gly Thr Leu Val Thr Val Ser Ser
115            120

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What is claimed is:

1. A molecule comprising the antigen-binding portion of an isolated antibody which has an increased affinity for a receptor protein tyrosine kinase and which blocks constitutive activation of said receptor protein tyrosine kinase.

2. The molecule according to claim 1, wherein said molecule binds to the extracellular domain of said receptor protein tyrosine kinase.

3. The molecule according to claim 1 wherein the antibody binds the dimeric form of the receptor.

4. The molecule according to claim 1, wherein the receptor protein tyrosine kinase is selected from the group consisting of EGFR/ErbB1, ErbB2/HER2/Neu, ErbB/HER3, ErbB4/HER4, IGF-1R, PDGFR- α , PDGFR- β , CSF-1R, kit/SCFR, Flk2/FH3, Flk1/VEGFR1, Flk1/VEGFR2, Flt4/VEGFR3, FGFR1, FGFR2/K-SAM, FGFR3, FGFR4, TrkA, TrkC, HGFR, RON, EphA2, EphB2, EphB4, Axl, TIE/TIE1, Tek/TIE2, Ret, ROS, and Alk, and heterodimeric combinations thereof.

5. The molecule according to claim 4, wherein said receptor protein tyrosine kinase is a fibroblast growth factor receptor (FGFR).

6. The molecule according to claim 5, wherein said FGFR is FGFR3.

7. The molecule according to claim 1, comprising a V_H region and a V_L region, respectively, selected from SEQ ID NO:103 and SEQ ID NO:92; SEQ ID NO:105 and SEQ ID NO:94; and SEQ ID NO:113 and SEQ ID NO:102.

8. The molecule according to claim 1, comprising a V_H -CDR3 region and a V_L -CDR3 region, respectively, selected from SEQ ID NO: 8 and SEQ ID NO: 9; SEQ ID NO: 12 and SEQ ID NO:13; and SEQ ID NO: 24 and SEQ ID NO:25.

9. The molecule according to claim 8, comprising a V_H -CDR3 region and a V_L -CDR3 region having SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:84, SEQ ID NO:89 and SEQ ID NO:91 or a polynucleotide sequence hybridizing under high stringency conditions thereto.

10. A pharmaceutical composition, comprising as an active ingredient at least one molecule according to claim 1 and a pharmaceutically acceptable carrier, excipient, or auxiliary agent.

11. An isolated nucleic acid molecule, comprising a polynucleotide sequence selected from SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:84, SEQ ID NO:89 and SEQ ID NO:91 or a polynucleotide sequence hybridizing under high stringency conditions thereto.

12. An isolated nucleic acid molecule, comprising polynucleotide sequences encoding a V_H region and a V_L region, respectively, selected from SEQ ID NO: 84 and SEQ ID NO:74; SEQ ID NO: 89 and SEQ ID NO:75; and SEQ ID NO: 91 and SEQ ID NO:76 or a polynucleotide sequence hybridizing under high stringency conditions thereto.

13. An isolated nucleic acid molecule, comprising a polynucleotide sequence selected from SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:50 and SEQ ID NO:51 or a polynucleotide sequence hybridizing under high stringency conditions thereto.

14. An isolated nucleic acid molecule, comprising polynucleotide sequences encoding a V_H -CDR3 region and a V_L -CDR3 region, respectively, selected from SEQ ID NO:30 and SEQ ID NO:31; SEQ ID NO: 34 and SEQ ID NO:35 and SEQ ID NO:50 and SEQ ID NO:51 or a polynucleotide sequence hybridizing under high stringency conditions thereto.

15. A molecule comprising the antigen-binding portion of an antibody which binds to a fibroblast growth factor receptor (FGFR) and which blocks ligand-dependent activation of the FGFR.

16. The molecule according to claim 15, wherein said molecule binds to the extracellular domain of said FGFR.

17. The molecule according to claim 16, wherein said FGFR is FGFR3.

18. The molecule according to claim 15, comprising a V_H region and a V_L region, respectively, selected from SEQ ID NO:104 and SEQ ID NO:93; SEQ ID NO:106 and SEQ ID NO:95; SEQ ID NO:107 and SEQ ID NO:96; SEQ ID NO:108 and SEQ ID NO:97; SEQ ID NO:109 and SEQ ID NO:98; SEQ ID NO:110 and SEQ ID NO:99; SEQ ID NO:111 and SEQ ID NO:100 and SEQ ID NO:112 and SEQ ID NO:101.

19. A nucleic acid molecule according to claim 18, wherein the nucleic acid molecule is in a vector or a host cell comprising a vector.

20. The molecule according to claim 15, comprising a V_H -CDR3 region and a V_L -CDR3 region selected from SEQ ID NO:10 and SEQ ID NO:11; SEQ ID NO:14 and SEQ ID NO:15; SEQ ID NO:16 and SEQ ID NO:17; SEQ ID NO:18 and SEQ ID NO:19; SEQ ID NO:20 and SEQ ID NO:21; SEQ ID NO:22 and SEQ ID NO:23; SEQ ID NO:26 and SEQ ID NO:27 and SEQ ID NO:28 and SEQ ID NO:29.

21. A nucleic acid molecule according to claim 20, wherein the nucleic acid molecule is in a vector or a host cell comprising a vector.

22. A pharmaceutical composition, comprising as an active ingredient at least one molecule according to claim 15 and a pharmaceutically acceptable carrier, excipient, or auxiliary agent.

23. An isolated nucleic acid molecule, comprising a polynucleotide sequence selected from SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:78, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:86 and SEQ ID NO:87 or a polynucleotide sequence hybridizing under high stringency conditions thereto.

24. A nucleic acid molecule according to claim 23, wherein the nucleic acid molecule is in a vector or a host cell comprising a vector.

25. An isolated nucleic acid molecule, comprising polynucleotide sequences encoding a V_H region and a V_L region, respectively, selected from SEQ ID NO:85 and SEQ ID NO:70; SEQ ID NO:78 and SEQ ID NO:67; SEQ ID NO:79 and SEQ ID NO:64; SEQ ID NO:86 and SEQ ID NO:71; SEQ ID NO:80 and SEQ ID NO:62; SEQ ID NO:87 and SEQ ID NO:65; SEQ ID NO:82 and SEQ ID NO:73 and SEQ ID NO:83 and SEQ ID NO:69 or a polynucleotide sequence hybridizing under high stringency conditions thereto.

26. A nucleic acid molecule according to claim 25, wherein the nucleic acid molecule is in a vector or a host cell comprising a vector.

27. An isolated nucleic acid molecule, comprising a polynucleotide sequence selected from SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48 and SEQ ID NO:49 or a polynucleotide sequence hybridizing under high stringency conditions thereto.

28. A nucleic acid molecule according to claim 27, wherein the nucleic acid molecule is in a vector or a host cell comprising a vector.

29. An isolated nucleic acid molecule, comprising polynucleotide sequences encoding a V_H -CDR3 DNA region and a V_L -CDR3 DNA region, respectively, selected from SEQ ID NO:32 and SEQ ID NO:33; SEQ ID NO:36 and SEQ ID NO:37 and SEQ ID NO:38 and SEQ ID NO:39; SEQ ID NO:40 and SEQ ID NO:41; SEQ ID NO:42 and SEQ ID NO:43; SEQ ID NO:44 and SEQ ID NO:45; SEQ ID NO:46 and SEQ ID NO:47 and SEQ ID NO:48 and SEQ ID NO:49 or a polynucleotide sequence hybridizing under high stringency conditions thereto.

30. A nucleic acid molecule according to claim 29, wherein the nucleic acid molecule is in a vector or a host cell comprising a vector.

31. A kit comprising the molecule of claim 1 or a molecule comprising the antigen-binding portion of an antibody which binds to a fibroblast growth factor receptor (FGFR) and which blocks ligand-dependent activation of the FGFR, the kit further comprising at least one reagent suitable for detecting the presence of said molecule when bound to said receptor protein tyrosine kinase and instructions for use.

32. A method for treating or inhibiting a skeletal dysplasia or a craniosynostosis disorder, comprising administering a therapeutically effective amount of the pharmaceutical composition to a subject in need thereof, wherein the pharmaceutical composition is according to claim 10 or is a pharmaceutical composition comprising as an active ingredient at least one molecule that is an antigen-binding portion of an antibody which binds to a fibroblast growth factor receptor (FGFR) and which blocks ligand-dependent activation of the FGFR.

33. The method according to claim 32, wherein the skeletal dysplasia is selected from achondroplasia, thanatophoric dysplasia (TD), hypochondroplasia, severe achondroplasia with developmental delay and acanthosis nigricans (SADDAN) dysplasia.

34. The method according to claim 33, wherein said skeletal dysplasia is achondroplasia.

35. The method according to claim 32, wherein the craniosynostosis disorder is Muenke coronal craniosynostosis or Crouzon syndrome with acanthosis nigricans.

36. The method according to claim 32, wherein the disorder is associated with constitutive activation of a receptor protein tyrosine kinase or with ligand-dependent activation of a receptor protein tyrosine kinase.

37. The method according to claim 36, wherein the receptor protein tyrosine kinase is FGFR3.

38. A method for treating or inhibiting a cell proliferative disease or disorder associated with abnormal RPTK activity, comprising administering a therapeutically effective amount of the pharmaceutical composition to a subject in need thereof, wherein the pharmaceutical composition is according to claim 10 or is a pharmaceutical composition comprising as an active ingredient at least one molecule that is an antigen-binding portion of an antibody which binds to a fibroblast growth factor receptor (FGFR) and which blocks ligand-dependent activation of the FGFR.

39. The method according to claim 38, wherein the cell proliferative disease or disorder is selected from solid tumors, non-solid cancer or tumor progression,

40. The method according to claim 39, wherein the tumor progression is the progression of transitional cell carcinoma, mammary carcinoma, osteosarcoma or chondrosarcoma.

41. The method according to claim 39, wherein the non-solid cancer is a hematopoietic malignancy.

42. The method according to claim 41, wherein the hematopoietic malignancy is multiple myeloma.

43. The method according to claim 38, wherein the disorder is associated with the action of a constitutively activated receptor protein tyrosine kinase or with ligand-dependent activation of a receptor protein tyrosine kinase.

44. A method for screening a molecule comprising the antigen-binding portion of an antibody which blocks ligand-independent or ligand-dependent activation of a receptor protein tyrosine kinase, comprising:

providing a library of antigen binding fragments;

screening a library of antigen binding fragments for binding to a dimeric form of a receptor protein tyrosine kinase;

identifying an antigen binding fragment which binds to the dimeric form of the receptor protein tyrosine kinase as a candidate molecule for blocking constitutive activation of the receptor protein tyrosine kinase; and

determining whether the candidate molecule blocks constitutive and or ligand-dependent activation of the receptor protein tyrosine kinase in a cell.

45. The method according to claim 44, wherein said receptor protein tyrosine kinase is selected from the group consisting of EGFR/ErbB1, ErbB2/HER2/Neu, ErbB/HER3, ErbB4/HER4, IGF-1R, PDGFR- α , PDGFR- β , CSF-1R, kit/SCFR, Flk2/FH3, Flk1/VEGFR1, Flk1/VEGFR2, Flt4/VEGFR3, FGFR1, FGFR2/K-SAM, FGFR3, FGFR4, TrkA, TrkC, HGFR, RON, EphA2, EphB2, EphB4, Axl, TIE/TIE1, Tek/TIE2, Ret, ROS, and Alk, and heterodimeric combinations thereof.

46. The method according to claim 45, wherein said receptor protein tyrosine kinase a fibroblast growth factor receptor.

47. The method according to claim 46, wherein said fibroblast growth factor receptor is FGFR3.

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